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PASTURE STUDIES XXIV.

VIABLE SEEDS IN PASTURE SOIL AND MANURE

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The flora of any pasture exhibits considerable variation from time to time both in the occurrence and in the abundance of its component species due to various causes. The increase in the area covered by a species or the advent of a new species may come about by two means: first, by extension from plants already present by means of vegetative structures which invade and take possession of vacant space or space occupied by weaker growing plants; and, second, by the establishment of new plants from seed.

The first of these methods is, by far, the most important in old permanent pastures with firmly established sod and where continuous close grazing prevents the development of seed-producing stalks. The growth-points and perennating organs, on which these plants depend for survival and spread, are borne in unexposed positions near the surface of the ground or just beneath it on rhizomes, stolons or basal portions of upright stems.

Some of the commonest and most desirable species, such as *Poa pratensis*, *Agrostis stolonifera*, *Festuca rubra*, *Poa compressa*, and *Agropyron repens*, as well as species of *Carex* and *Scirpus*, *Rumex Acetosella* and *Achillea Millefolium* spread extensively by sub-surface rhizomes. Stolons, which equip the plant for more rapid extensive spread than rhizomes, although establishment therefrom may not be as secure, are possessed by *Trifolium repens*, probably the most valuable of all plants in permanent pastures, and several other common forms such as *Hieracium aurantiacium*, *Cerastium vulgatum*, *Veronica serpyllifolia*, *Stellaria graminea*, *Viola pallens*, etc. In some situations certain bent-grasses (*Agrostis*) carpet the ground with leafy stolons. The bulbous bases of *Phleum pratense*, decumbent caudices of *Chrysanthemum Leucanthemum*, *Prunella vulgaris*, and *Solidago nemoralis*, geniculate stems of *Panicum lanuginosum*, rosette offsets of *Erigeron philadelphicus* and *Antennaria neglecta* and the dividing tap-root of *Taraxacum officinale*, are further examples of vegetative structures fitting plants to existence under grazing conditions. Upright plants with terminal growing points cannot survive.

Propagation by means of seeds may also become a potent factor in the establishment of plants especially in the covering over of bare spaces. Seeds reaching the ground become worked into the surface soil by earthworms, trampling of the stock or heaving and cracking of the earth on

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freezing or on drying (3). Here they may remain for long periods of time, retaining their viability, before germinating (5). There is, however, great variability in the longevity of seeds of different species buried in the soil (6). Some, if they do not germinate immediately on maturity or soon afterwards, lose their vitality, while others exhibit dormancy and will not grow until the following season or periodic intervals thereafter (2).

In pasture stands, the seeds, if they are able to mature at all, fall to the ground in the majority of species in the immediate vicinity of the base of the parent plant. Some, however, are mobile by reason of specialized appendages or are dependent on some mechanism or external agency for their dispersal into more distant areas. For example, wind becomes the dispersive agent for plumed seeds such as those found in the pasture composites, *Taraxacum officinale*, *Hieracium aurantiacum* and *florentinum*, *Antennaria neglecta*, *Leontodon autumnalis*, *Erigeron* spp., *Cirsium* spp. and *Solidago* spp. Water, other than slight surface wash by heavy rains, is not important in carrying seeds of pasture species. The seeds of these, moreover, are rarely buoyant. No plants with hooked or barbed fruits (with the exception of the rare individual of *Geum*) are found in pastures so that transportation by adhering to animals' hair is not as important as might be expected. Explosive mechanisms are provided by such plants as *Oxalis europaea*, *Prunella vulgaris* and *Vicia Cracca* for dissemination of their seeds for distances of a few inches only.

Many species, however, produce seeds that are able to withstand digestion and will pass unharmed through the alimentary tract of cattle (10). This method of distribution has been studied mainly from the standpoint of the spread of weeds by manuring (1, 7). Some observations have been made on the spread of seeds, mainly of *Trifolium repens*, by grazing animals (8), but actual investigations of the possibilities of forage species invading permanent pastures by this route are very scant. In order to obtain more information on natural reseeding, this experiment was devised in connection with the Macdonald College investigations in the Eastern Townships of Quebec (9), to test the viability of seeds in soil and cattle droppings in permanent pastures.

EXPERIMENTAL METHOD

A. *Soil Samples*

Sections of sod and surface soil, 6 × 6 inches in size and 1 inch deep, were cut from unfertilized areas in four selected permanent pastures in the vicinity of Cowansville, Que. Four samples were taken at each pasture in the latter part of June when very few plants of the season had gone to seed, and a second set in contiguous positions, in September when fruiting was mainly completed. Botanical estimates were made of the cover of each sample and of the ground in the immediate vicinity. An approximation to the ground covered by each species of plant based on quadrat readings on unfertilized areas is given for each of the pastures in Table 1.

Each sample was treated separately; dried, broken up, stems and roots removed and thoroughly mixed. One quarter of the material, which represented 9 square inches of pasture surface, was spread in a thin layer on the

surface of sterilized soil in the greenhouse in late October. A duplicate set started in January was moistened, then alternately frozen and thawed three times before planting.

B. *Manure Samples*

Fresh cattle droppings were collected at two of the pastures in early, middle and late summer and allowed to dry at air temperatures. Notes were made on the plants in fruit at each period.

When thoroughly dry the manure was finely broken up and the four samples from each collection composited. Five-ounce portions of these composites were spread on sterilized soil in flats in the greenhouse. A second set was given a freezing treatment similar to the soil samples.

All flats were watered in order to provide conditions suitable for germination. When the seedlings began to appear they were identified, then counted and removed from the flats at intervals. Although germination was slow during the three or four winter months while the experiment was in progress, most of the samples showed seedlings within a few weeks after setting out. Apart from its unsuitability for germination, the winter was found to be an ideal time for testing since very few seeds are then floating in the air to enter the greenhouse and contaminate the tests.

TABLE 1.—PERCENTAGE GROUND COVERED BY SPECIES OF PLANTS ON UNFERTILIZED AREAS OF THE FOUR PERMANENT PASTURES IN THE VICINITY OF COWANSVILLE, QUE.

Species	Pasture				Average
	A	B	C	D	
Agrostis stolonifera	20.6	10.6	20.3	24.6	19.3
Festuca rubra	—	35.6	2.1	1.4	9.8
Hieracium aurantiacum	12.4	1.7	14.4	+	7.2
Trifolium repens	5.3	10.9	0.8	9.4	6.6
Phleum pratense	8.7	2.1	9.5	4.4	6.2
Taraxacum officinale	6.7	1.2	+	13.3	5.3
Poa pratensis	4.0	2.0	+	6.7	3.2
Danthonia spicata	+	3.6	8.1	+	3.0
Plantago major	0.8	0.6	1.3	5.6	2.1
Ranunculus acris	2.1	0.8	0.5	5.0	2.1
Prunella vulgaris	2.4	1.4	1.3	2.1	1.8
Panicum lanuginosium	1.7	+	4.8	+	1.7
Fragaria virginiana	1.6	1.8	2.6	+	1.5
Carex spp.	3.2	+	1.5	0.6	1.4
Oxalis europaea	1.1	2.2	1.4	+	1.3
Chrysanthemum Leucanthemum	+	1.9	1.8	+	1.1
Potentilla simplex	—	+	4.0	+	1.1
Cyperus diandrus	+	—	—	3.9	1.0
Solidago spp.	+	+	1.7	+	0.7
Viola pallens	1.1	0.7	+	0.6	0.6
Digitaria Ischaemum	+	2.5	—	—	0.6
Hieracium florentinium	1.3	+	0.5	—	0.5
Achillea Millefolium	+	1.7	+	+	0.5
Juncus macer	0.9	+	+	0.7	0.4
Antennaria neglecta	0.7	0.7	+	+	0.4
Linum catharticum	—	—	—	1.4	0.4
Poa compressa	0.5	+	+	0.6	0.3
Glyceria striata	0.5	—	—	—	0.2
Stellaria graminea	+	—	0.6	—	0.2
Trifolium agrarium	—	+	0.7	+	0.2

TABLE 1.—PERCENTAGE GROUND COVERED BY SPECIES OF PLANTS ON UNFERTILIZED AREAS OF THE FOUR PERMANENT PASTURES IN THE VICINITY OF COWANSVILLE, QUE.—*Concluded*

Species	Pasture				Average
	A	B	C	D	
Cerastium vulgatum	+	+	+	+	0.1
Veronica serpyllifolia	+	+	+	+	0.1
Cirsium spp.	+	—	+	+	+
Hedeoma pulegioides	+	—	—	—	+
Lycopus spp.	+	+	+	+	+
Oenothera perennis	+	+	+	+	+
Potentilla norvegica	+	+	+	+	+
Sisyrinchium angustifolium	+	+	+	+	+
Spiraea tomentosa	+	+	+	—	+
Sporobolus neglectus	+	—	—	—	+
Vicia Cracca	—	—	+	—	+
Trifolium pratense	—	+	+	+	+
Leontodon autumnalis	+	+	—	+	+
Rumex Acetosella	+	+	+	+	+
Hypericum spp.	+	+	+	+	+
Festuca elatior	+	+	+	+	+
Hydrocotyle americana	+	+	+	+	+
Agropyron repens	+	—	+	—	+
Equisetum arvense	+	—	—	+	+
Echinochloa crusgalli	+	—	—	—	+
Erigeron sp.	+	+	+	+	+
Galium sp.	+	+	+	+	+
Lobelia inflata	+	+	—	—	+
Plantago lanceolata	—	+	—	—	+
Veronica officinalis	—	+	—	—	+
Setaria lutescens	—	—	+	—	+

Plants present in less than 0.5% of ground cover are indicated by +.
The pastures selected for sampling were on the farms of the following persons: A. Mr. W. R. Beach; B. Mr. L. M. Doherty; C. Mr. W. E. Dryden; D. Mr. A. Robertson.

RESULTS OF GERMINATION TESTS

A. *Soil Samples*

The number of seedlings arising from viable seeds contained in the samples of surface soil from the four permanent pastures are given in Table 2. The data in this table are considerably condensed, so that each figure indicates the number of seedlings growing from the total of four June and four September samples. Since each sample represented 9 square inches of pasture surface and was tested in duplicate, the number of seedlings given are those that may be expected from every square foot ($8 \times 9 \times 2 = 144$ sq. in.) of pasture. For comparison the average for the district in thousands per acre is given in the right-hand column.

TABLE 2.—SEEDLINGS ARISING FROM VIABLE SEEDS IN SURFACE SOIL FROM PERMANENT PASTURES

Species	Number of seedlings per sq. ft. of pasture surface				Average number per acre (thousands)*
	Pasture				
	A	B	C	D	
Cyperus diandrus	56	—	—	366	4596
Juncus macer	88	15	97	85	3104
Danthonia spicata	—	108	39	1	1612
Chrysanthemum Leucanthemum	1	2	115	—	1280
Cerastium vulgatum	16	—	28	51	1045
Panicum lanuginosum	21	—	59	—	871

TABLE 2.—SEEDLINGS ARISING FROM VIABLE SEEDS IN SURFACE SOIL FROM PERMANENT PASTURES—*Concluded*

Species	Number of seedlings per sq. ft. of pasture surface				Average number per acre (thousands)*
	Pasture				
	A	B	C	D	
Agrostis stolonifera	28	7	24	10	750
Plantago major	14	1	2	40	621
Potentilla norvegica	6	16	19	15	610
Erigeron ramosus	—	—	54	1	599
Hypericum spp.†	31	—	4	14	534
Digitaria Ischaemum	—	31	—	8	425
Lobelia inflata	9	9	1	12	338
Oenothera pumila‡	6	1	17	5	316
Poa compressa	13	4	—	7	261
Oxalis europaea	4	1	10	2	185
Rumex Acetosella	1	—	16	—	185
Veronica serpyllifolia	12	1	—	4	185
Linum catharticum	—	—	—	17	185
Echinochloa crusgalli	2	1	8	3	152
Viola pallens	1	8	—	—	98
Chenopodium album	1	5	2	—	87
Trifolium repens	2	1	—	5	87
Hieracium aurantiacum	1	—	6	—	76
Solidago spp.	—	—	2	—	76
Phleum pratense	—	—	4	2	65
Prunella vulgaris	1	1	—	4	65
Poa pratensis	3	—	—	2	54
Carex spp.	1	—	2	1	44
Lychnis alba	—	—	4	—	44
Verbascum Thapsus	3	—	—	1	44
Antennaria neglecta	1	2	—	—	33
Anthoxanthum odoratum	—	2	1	—	33
Hedeoma pulegioides	3	—	—	—	33
Setaria viridis	—	—	2	—	33
Stellaria graminea	—	—	3	—	33
Capsella Bursa-pastoris	1	1	—	—	22
Cirsium lanceolatum	—	—	1	1	22
Fragaria virginiana	1	—	1	—	22
Glyceria striata	—	—	—	2	22
Lycopus americanus	—	—	—	2	22
Setaria lutescens	—	—	2	—	22
Sisyrinchium angustifolium	—	—	2	—	22
Taraxacum officinale	—	—	—	2	22
Achillea Millefolium	—	1	—	—	11
Aster sp.	1	—	—	—	11
Erigeron annuus	1	—	—	—	11
Galeopsis Tetrahit	—	—	1	—	11
Geum sp.	—	—	1	—	11
Poa annua	1	—	—	—	11
Polygonum aviculare	—	—	—	1	11
Ranunculus acris	—	—	—	1	11
Sambucus sp.	—	—	1	—	11
Spiraea tomentosa	—	—	1	—	11
Trifolium pratense	—	—	—	1	11
Vicia Cracca	1	—	—	—	11
Unidentified grasses§	3	—	—	—	33
Unidentified forbs§	8	—	7	3	196
Total number seedlings	342	218	537	675	19297
Number of species	32	21	33	32	57

* Figures previously published were incorrectly calculated from same data (9).
† Includes *H. mutilum* and *H. canadense*.
‡ Includes a few seedlings of *Epilobium* sp.
§ Not mature enough at conclusion of experiment for certain identification.

From the results obtained in the germination tests, we find that the surface soil of permanent pastures is a substantial storehouse of viable seeds. An average figure for the district shows about 19,297,000 potential plants per acre. This is slightly more than the estimated number of living plants that form the sward of average pasturelands[3].

One of the strange features is that the majority of the seeds are of species (*Agrostis stolonifera* excepted) not found in large quantity in any of the permanent pastures. The five species that contribute 63% of all the seeds, contribute less than 7% to the herbage cover of the pasture. Of the six commonest plants, *Agrostis stolonifera*, *Festuca rubra*, *Hieracium aurantiacum*, *Trifolium repens*, *Phleum pratense* and *Taraxacum officinale*, each of which covers over 5% of the ground, only one, *Agrostis*, is well represented by seeds with about 4% of the total. The others made up slightly over 0.5%; seeds of *Festuca rubra* not being found at all.

Of the 57 species of seedlings, 12 were annuals, 4 were biennials, and 26 were either short-lived, non-stoloniferous, or non-rhizomatous perennials. These are the types of plants that would be most susceptible to extermination by close grazing and owe their existence to the abundance and longevity of their seeds. Vigorous perennials such as *Juncus macer*, *Cerastium vulgatum*, *Panicum lanuginosum*, *Plantago major*, *Oxalis europaea* and *Veronica serpyllifolia* which produce their flowers and fruits close to the surface of the ground are well represented by seeds in the soil. Cleistogamous flowers borne, as they are in *Danthonia spicata*, in the basal sheaths or on the surface of the soil, in the case of *Viola*, give rise to seeds which are probably more essential to the persistence of these species than seeds formed in the conspicuous inflorescences.

It is also surprising to note the absence or relative paucity of species which produce an abundance of seeds suited for efficient wind dissemination as *Taraxacum officinale*, *Hieracium aurantiacum*, *Leontodon autumnalis*, *Erigeron* and *Solidago*. There is the possibility that the seeds of these may be able to germinate only for a short period after they are shed and seedlings, if they cannot establish then, will not be produced later on. Seeds of several of the valuable forage species, *Trifolium repens*, *Poa pratensis*, *Phleum pratense* and *Festuca rubra* are in small amount or absent. *Agrostis stolonifera*, however, is prevalent about equally in all samples. Some of the individual samples, nevertheless, were taken in areas in the pastures often covered 75% by these species. The correlation, if any, between the seed flora of the soil samples and the herbage flora appears to be a negative one.

The presence of seeds in the samples, on the other hand, corresponds well with the presence of plants in the herbage of the pasture taken as a whole. Comparison of the figures in Table 1 and Table 2 shows that in the majority of cases if a species is scarce or absent in the flora of the pasture it is generally represented by few or no seeds in the soil.

The number of seeds in the samples collected in early summer and in late summer was not greatly different. Freezing of the samples prior to planting had no noticeable effect on germination.

[3] 16,790,000 is the average for 10 pastures in Britain as reported by Armstrong. Jour. Agr. Sci. 2: 302-303. 1907.

B. *Manure Samples*

The seedlings arising from seeds that must have been eaten, subjected to mastication and digestion, and voided by the cattle at different periods in the season are given in Table 3 for the two pastures from which collections were made. The figures are the totals for the duplicate tests. The estimated number of seeds carried into the pasture by each cow is calculated from the average of the figures, taking 6.5 pounds as the general daily dry matter defecation based on the work of Crampton and Purdy (4). During the summer months the cattle received no supplementary feed and we may assume that all the seeds that were found in the manure were picked up by the cattle in the pastures or in near-by places where they had had an opportunity to graze. The species found in the manure give evidence to the fact that the stock must have browsed along roadways and in the barnyards as well as on the aftermath of hay meadows.

TABLE 3.—SEEDLINGS ARISING FROM VIABLE SEEDS IN MANURE FROM PERMANENT PASTURES AT PERIODS DURING THE SEASON

Species	Number of seedlings per 10 oz. of dried cattle manure						Number distributed by a cow in 165-day grazing season (thousands)
	Pasture B			Pasture D			
	June 20	Aug. 6	Sept. 10	July 20	Aug. 15	Sept. 10	
Agrostis stolonifera	—	237	118	259	91	44	214.2
Phleum pratense	18	39	247	25	47	55	151.9
Poa pratensis	1	199	12	132	17	29	111.5
Trifolium repens	3	161	25	67	42	19	90.7
Chenopodium album	—	—	245	—	—	6	71.8
Poa compressa	2	65	24	85	33	28	67.8
Cerastium vulgatum	65	15	2	7	5	—	26.9
Carex spp.	—	7	14	51	19	—	26.0
Plantago major	2	2	64	3	5	15	26.0
Veronica serpyllifolia	57	2	4	2	—	—	18.6
Danthonia spicata	5	11	15	17	4	—	14.9
Ranunculus acris	—	4	14	9	6	—	9.4
Plantago lanceolata	—	7	7	15	—	—	8.3
Juncus macer	1	2	12	2	6	—	6.6
Rumex Acetosella	—	7	4	12	—	—	6.6
Amaranthus retroflexus	—	—	21	—	—	—	6.0
Veronica officinalis	—	—	8	9	1	1	5.4
Poa annua	6	6	2	1	2	1	5.1
Echinochloa crusgalli	—	2	9	3	—	1	4.3
Festuca elatior	—	8	2	3	—	—	3.7
Festuca rubra	4	6	—	3	—	—	3.7
Panicum lanuginosum	—	—	1	4	5	3	3.7
Potentilla norvegica	—	2	—	—	—	8	2.9
Setaria lutescens	—	—	6	—	—	2	2.3
Glyceria striata	—	2	—	3	—	—	1.4
Fragaria virginiana	2	—	—	2	—	—	1.1
Lychnis alba	—	1	2	—	1	—	1.1
Potentilla simplex	—	4	—	—	—	—	1.1
Prunella vulgaris	—	—	1	1	1	—	1.1
Trifolium pratense	—	1	1	1	—	—	1.1
Polygonum Hydropiper	—	—	3	—	—	—	.9
Trifolium hybridum	—	2	—	—	—	—	.9
Trifolium procumbens	—	—	—	—	1	2	.9
Digitaria Ischaemum	1	—	1	—	—	—	.6
Galium sp.	—	—	1	1	—	—	.6
Trifolium agrarium	—	2	—	—	—	—	.6

TABLE 3.—SEEDLINGS ARISING FROM VIABLE SEEDS IN MANURE FROM PERMANENT PASTURES AT PERIODS DURING THE SEASON—*Concluded*

Species	Number of seedlings per 10 oz. of dried cattle manure						Number distributed by a cow in 165-day grazing season (thousands)
	Pasture B			Pasture D			
	June 20	Aug. 6	Sept. 10	July 20	Aug. 15	Sept. 10	
Acalypha virginica	—	—	1	—	—	—	.3
Anthoxanthum odoratum	—	1	—	—	—	—	.3
Avena sativa	—	—	1	—	—	—	.3
Chrysanthemum Leucanthemum	—	—	1	—	—	—	.3
Cirsium lanceolatum	—	—	—	1	—	—	.3
Geum sp.	—	—	—	—	—	1	.3
Muhlenbergia foliosa	—	—	—	—	—	1	.3
Polygonum Convolvulus	—	—	—	—	—	1	.3
Polygonum pennsylanicum	—	—	—	—	—	1	.3
Polygonum Persicaria	—	—	—	—	1	—	.3
Rumex crispus	—	—	—	—	—	1	.3
Silene latifolia	—	—	1	—	—	—	.3
Setaria viridis	—	—	1	—	—	—	.3
Total number of seedlings	167	795	970	719	297	212	903.8
Number of species	13	26	33	27	19	19	49

Probably the outstanding result of this experiment was the finding that cattle are the most important of the various agencies for the dispersal of seeds of the pasture species. From the calculations, we find that a single cow may distribute on the average about 903,760 seeds in its manure during the length of the grazing season. Among the seeds so distributed, the desirable forage species are well represented. *Trifolium repens*, *Poa pratensis*, *Agrostis stolonifera*, *Phleum pratense* and *Poa compressa* are probably some of the most valuable of all the pasture plants of the district, and their seeds are present in the manure in numbers making up 70% of all seeds found. The fruiting parts of these plants are upright and easily accessible, and the seeds produced thereon are evidently quite resistant to digestion. Seeds of *Festuca rubra*, a palatable and abundant species o the district also, however, are not found. It is known that *Trifolium repens* produces "hard seeds" and it is probably on this account that they are able to withstand injury on passing through the alimentary tract of the cattle. Clover seedlings are commonly found growing from the old droppings in the pastures. In some countries it is the practice of graziers to turn their stock into a field where the clover plants are in fruit and then drive them on to land that requires reseeding with this species. The number of seedlings of *Trifolium* that came up in the samples, although of considerable number, is not a complete measure of the total viable seeds that were present in the manure. A large number of apparently firm and good seeds of this species were found to be present in some of the samples even at the time when it was necessary to discontinue the germination experiment.

Unlike the soil, the seasonal flora of manure is very striking. Comparatively few seeds ripen before the middle of June and consequently few are to be found in the droppings up to that time. *Cerastium vulgatum*,

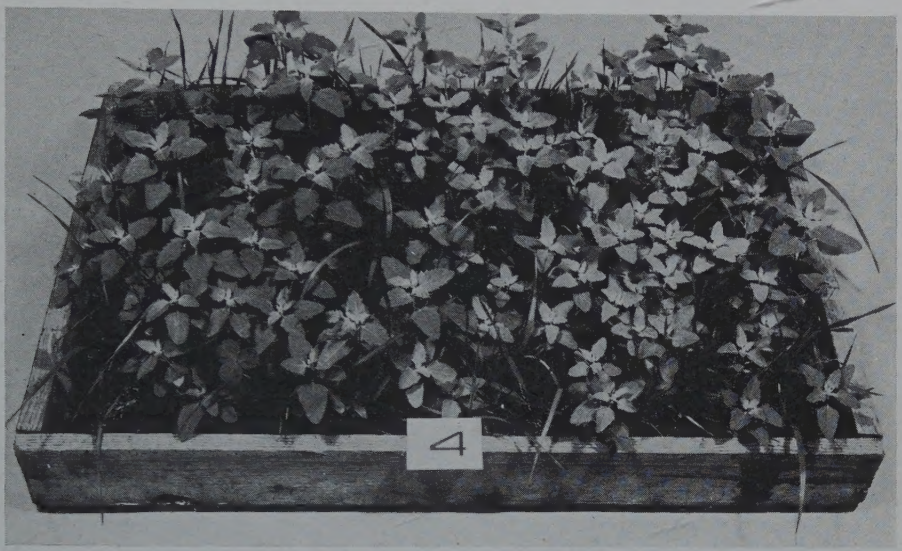

FIGURE 1. Seedlings growing from viable seeds in the manure deposited in Pasture B on September 10. The 109 seedlings of *Chenopodium album* obscure the presence of the other species in the germination flat.

FIGURE 2. The same flat after removal of the *Chenopodium* to show the other species, *Trifolium pratense*, *Rumex Acetosella*, *Ranunculus acris*, *Plantago major*, *Cerastium vulgatum*, etc. The total number of seedlings growing from this sample during the six-month test are given in Table 3.

Veronica serpyllifolia, *Poa annua*, *Fragaria virginiana*, *Antennaria neglecta*, and *Viola pallens* were the only species recorded in fruit on June 20. Seeds of the first four of these are present in considerable number in the manure. Where the few seedlings of *Phleum pratense*, *Danthonia spicata*, *Festuca rubra* and some of the later fruiting species came from, is open to speculation. Obviously, some dried fruiting panicles or seed heads, or in the case of *Danthonia* the basal cleistogamous seeds, remaining on the pasture from the previous year must have been picked up. The mid-season collections caught the majority of the plants at the height of fruiting. Numerous seedlings grew from manure deposited by cattle from July 15 onwards. In september the late maturing species, particularly the annuals, occur. Among those remaining viable after digestion are *Chenopodium album*, *Amaranthus retroflexus*, *Echinochloa crusgalli*, *Setaria lutescens* and *Digitaria Ischaemum*. Figure 1 shows the two-months old seedlings from 5 ounces of the dried manure from Pasture B on September 10. Figure 2 is the same germination test after the removal of the 109 seedlings of *Chenopodium album* which mask the appearance of the other species. Other seeds germinated as the test continued.

Later on in the season the number of seeds, particularly of some of the same annuals, increases still more. One particular sample collected in Pasture B in May, having been deposited in early November of the previous year, yielded the record number of seedlings. The list of the species from this single sample is given here. These figures multiplied by 20.8 will approximate the number distributed by each head of cattle each day

Chenopodium album	1,244
Amaranthus retroflexus	364
Phleum pratense	98
Echinochloa crusgalli	23
Trifolium pratense	19
Agrostis stolonifera	12
Digitaria Ischaemum	9
Plantago major	4
Polygonum Persicaria	4
Rumex Acetosella	3
Cerastium vulgatum	2
Stellaria media	2
Carex sp.	1
Juncus sp.	1
Plantago lanceolata	1
Poa annua	1
Setaria lutescens	1
Silene latifolia	1
Trifolium repens	1
Total number from 5 ounce sample	1,791
Average number distributed by one cow per day	37,253

towards the end of the grazing season. It was observed that the seedlings of *Amaranthus* were greatly deformed and mottled, tending to produce inflorescences when not more than an inch high and sometimes without the development of the first leaves. This abnormal state may have been due to injury caused the seeds by the digestive juices or to the conditions of germination, high fertility, moisture, short day, etc.

Species which are scarce or absent in the seed flora of the manure but which one might expect to find abundantly on account of their abundance in the pasture flora are *Festuca rubra*, *Taraxacum officinale*, *Prunella vulgaris*,

Panicum lanuginosum, *Oxalis europea*, *Chrysanthemum Leucanthemum*, *Antennaria neglecta*, *Cyperus diandrus*, etc. The seeds of these are presumably destroyed in digestion.

CONCLUSION

Although vegetative propagation is paramount in importance for the survival and spread of plants in closely and continuously grazed permanent pastures, seeds also play a significant part in the establishment of new plants and in the grassing over of bare spaces. Seeds lying dormant in the surface soil constitute a reserve of potential plants. These are mainly of annuals or weak-growing perennials which do not produce the typical vegetative structures and are not found in any great abundance in the pasture herbage. The seeds which are able to withstand animal digestion and are distributed in the manure of the cattle are another source from which new plants can enter the sward. The number of seeds dispersed in this manner is considerable but varies greatly from month to month depending on the stage of maturity of the pasture plants. The majority of the seeds are of the common and desirable forage species of grasses and legumes. Several of the generally occurring plants are not represented in the seed flora of the manure, their seeds presumably being unable to survive digestion.

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A NEW CONCEPT OF PURE SEED AS APPLIED TO SEED TECHNOLOGY[1]

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The concept of what constitutes "pure seed" has undergone modification from time to time during the course of seed testing history. Originally, all material of any species under consideration was counted as pure seed as long as it resembled a "seed" in external appearance, the word "seed" including seeds and fruits, with or without investing tissues, commonly classified as seeds in the trade. The method based on this definition has come to be known as the Irish method because it is still used in that country, at least as regards grass seeds. Later, some seed analysts felt that it was unscientific to class as seeds, structures in which this essential element was lacking, typically in undeveloped florets in grasses where only the glumes were present. Accordingly the definition of pure seed was changed to include only those structures containing a developed embryo. In the case of grasses, these were determined by stroking or pinching the seed or by examination on the diaphanoscope. This definition was adopted by the International Seed Testing Association and has been adhered to by the Association of Official Seed Analysts as applied to grasses. An excellent contribution toward clarifying the definition for grasses was that of Merl (12) in 1932. In the case of other kinds of seed, a cleavage of opinion was found to exist between the European and American workers, as a result of which two definitions were admitted, one for the so-called "Stronger Method" (S.M.) preferred by the former, the other for the "Quicker Method" (Q.M.) which is used by the latter.

According to the S. M. a pure seed must have a reasonable chance of developing a normal sprout, which means that an estimate of the viability of each seed must be made during the purity analysis. Since the seed is subjected to a subsequent germination test to determine this very point, the S. M. definition is considered inadmissible by American analysts.

The Q. M. uses a mechanical definition of pure seed according to which seeds larger than one-half are counted as pure seed and all portions one-half or less as inert matter, regardless of the condition of the embryo. This is considered equally inadmissible by European analysts, because portions of seeds may be placed in the inert fraction which yet might produce a normal plant thus fulfilling the essential function of a seed.

It would appear, then, that there is nothing final or absolute in the concept of what constitutes pure seed at the present time. The fact that practical difficulties lead to wide variations in results of analysis owing to differences in interpreting what ought to be classed as pure seed and what as inert matter, suggests that a re-examination of the whole position is needed.

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As far as grasses, in general, are concerned, there is little, if any, difference between the S. M. and Q. M. In both, the analyst must determine whether or not a caryopsis is present, preferably by means of transmitted light. The difficulty arises in determining where to put imperfectly developed caryopses. There is no clear line of demarcation and the variation in interpretation may amount to several per cent, although it is to be admitted that, in any one laboratory, by prolonged and careful training of analysts a satisfactory level of uniformity can be attained. Such uniformity, however, rarely extends to the results obtained on uniform material by different laboratories. The procedure, moreover, is time-consuming and a source of eye-strain even when aided by the use of a seed blower.

The rules for seed testing formulated by the Association of Official Seed Analysts define pure seed as all seeds of the kind in question over half in size regardless of condition, except that a seed of the Cruciferae or Leguminosae without a seed coat, and empty glumes in grasses, are considered inert material. Application of this definition of pure seed to the practice of seed testing is difficult in several classes of seeds, namely: (1) sweet clover, flax, sorghum, rye and other kinds with many broken seeds; (2) chaffy grasses whose fruiting glumes with no caryopsis are similar in appearance to those with a caryopsis; (3) insect injured seeds with part or all of the embryo destroyed yet with the seed coat intact; and (4) commercial seed lots of some flowers, vegetables, herbs, shrubs and trees in which few to many of the seeds or fruits are without an embryo. The large number of particles per gram of commercial seed in certain kinds of crops (5,000 to 9,000 in Kentucky bluegrass) prevents critical examination of each seed or particle which in turn increases the chance for error and for larger differences in interpretation than are to be expected from homogeneous samples. Difficulties inherent in the character of the material thus have been largely responsible for extreme differences in interpretation, which in turn have interfered with the orderly marketing of seeds.

The use of an air blast supplied either by a fan or compressed air has been of considerable assistance in the analysis of small seeded grasses and the attachment of a mercury gauge to read air pressure as proposed by Garman and carried out by Vaughan (3) marked an attempt to reduce personal errors. However, as was shown in a co-operative test conducted in 1937 (21) air blast separators employed by 6 laboratories were far from uniform in the separation from definitely heavy seed of stained particles known to be unfilled glumes. This test suggested that either the design of some of the old type separators was faulty or air pressures were not constant and that a pressure gauge could not correct such a fault.

In 1935 Brown and Porter (1) presented a paper before the Association of Official Seed Analysts outlining an improved method for the analysis of Kentucky bluegrass seed. The authors presented data showing extreme differences in purity obtained by 8 laboratories on a low and a high purity lot of bluegrass seed. Further, they described a proposed method, the basic principle of which was separation of unfilled glumes from pure seed by controlled, constant air pressure in a vertical tube. Their data indicated that by employing a constant speed motor and fan in conjunction with the Holland (Leendertz) air blast separator it was possible to obtain uniform separation of unfilled glumes from pure seed when the valve was opened to

the same point each time for a period of 10 minutes. The uniformity thus obtained with replicate subsamples from the same lot was far greater than that obtained with subsamples from the same original lot by 8 recognized laboratories using the official method. Finally, their data indicated that by increasing the flow of air a point was reached at which $\frac{\text{per cent pure} \times \text{per cent germination}}{100}$ gave a maximum index value. Stated in another way the data suggested that beyond a certain vent opening (increased pressure) the percentage germination of the pure seed was not increased but the purity was decreased which resulted in a decrease in index value of the lot. The separator unit employed removed a small number of viable seeds with the chaff for which a correction was made in percentage of pure seed. It is significant, however, that the correction did not materially change the relative index values of the samples blown at different pressures.

In August 1937, Porter (14), in a short paper, showed that percentages of pure seed obtained with 10 subsamples each from 2 lots of bluegrass, when analyzed by the improved method which employed a constant speed motor and fan, were uniform and the differences not highly significant.

In June 1938, Porter (15) presented additional data from 17 lots of bluegrass analyzed by the proposed method using from 4 to 10 subsamples from each lot. The results indicated that from only one lot (mean purity 96.7%) were the differences highly significant and in that case the difference between the high and low tests was only 1.00%. Germination tests of two separate pure seed fractions from each of 7 of the lots gave uniform results which further indicated the uniformity of operation by the air blast separator. In this same paper the author pointed out that chaffy grasses contain not only unfilled fruiting glumes but glumes with anthers only, or with shrivelled and undeveloped fruits light in weight and of low viability, the presence and percentage of which were determined with difficulty because of the large number of particles involved.

DEVELOPMENT OF NEW SEED SEPARATORS

In 1937, Leggatt (7) reported on a design for a new seed blower equipped with a manometer by means of which small differences in air pressure could be measured, and in June 1938, at the meeting of the Association of Official Seed Analysts he (8) presented data on the performance of the new blower. The results of his experiments showed that: (1) the percentage heavy seed obtained by repeated blowings of the same sample was quite uniform; (2) the mean weight of particle removed was increased with increase in pressure; (3) a 100 mesh, copper screen was superior to bolting cloth and also to a 73 mesh screen; and (4) as the weight of sample increased the pressure in the chamber necessary for removal of similar particles had to be increased.

At the same meeting Porter (16) described a new air blast separator quite different in construction from the one developed by Leggatt but his data on its performance were so similar that the two machines could well be expected to accomplish much the same purpose. The accuracy of separation seemed to be about equal. The data showed that with each increase in pressure above a certain point a larger percentage of florets was

viable. The data also showed the effect of size of sample on pressure readings and indicated that at a given valve opening, separation was accurate for samples ranging from 0.5 to 2 grams in weight even though the pressure in the compression chamber increased with size of sample. On the other hand it was shown that pressure in the separator tube was constant even though weight of sample varied. Porter's data included a study with synthetic samples containing 85% heavy seed and 15% empty florets stained red. Such samples were designed to serve as a standard for calibrating a separator equipped with a device for the control of air pressure. Uniformity and accuracy of separation were marked as shown by replicate blowings of the same sample.

The development of these two types of standardized blower, which can be calibrated to give highly uniform results, opened up the possibility of introducing a yet more refined uniform method.

These separate approaches to the problem of uniform and accurate separation of heavy and inert material in grasses were carried on more or less independently yet with some exchange of ideas. Since the basic principle involved was the same it was natural that both investigators should arrive at similar conclusions, namely, that a new concept of pure seed is needed and that a practical method of applying the principle to seed laboratory practice should be developed. It is the purpose of this paper to discuss these two conclusions.

STATISTICAL ESTIMATION OF VARIATION IN SEED SAMPLES AND OF THE ANALYTICAL METHODS EMPLOYED

In the early work with bluegrass it became evident that two problems required attention: (1) the degree of variation among subsamples that might be expected in homogeneous lots, and (2) the development of a method as free from error as possible that could be used to determine actual variability. By a study of these problems it was anticipated that differences could be measured and evaluated and further that a beginning might be made in determining the cause or causes for abnormal variations. Information on the two problems was obtained simultaneously.

Analytical error, that is, the discrepancy between the results of an analysis and the "true" value, is made up, broadly speaking and apart from mistakes, of two parts, sampling error and experimental error. The latter includes errors due to improper mixing, interpretational errors, weighing errors, and other errors that may occur during the course of an analysis. The former is due to lack of uniformity in the material itself; its limits are sharply defined and are an irreducible minimum, no matter how carefully mixing is done. When, in a statistical test of a series of data on the same bulk sample this minimum has been found to have been reached, it is reasonable to conclude that experimental errors have been virtually eliminated. Such a statistical test is the well-known chi-square test which has been extensively used by both authors in studying analytical variations. The results of the chi-square test are stated in terms of values of P, i.e., the probability that a series of values varying as greatly as those observed could result from a homogeneous lot of seed. If this probability

is small, say less than 1 : 100, then other factors than sampling error probably are causing the excessive variability.

On the other hand, caution should be exercised when the test gives extremely high values of P because $1 - P$ is the probability that a series of values varying as *little* as those observed could result from truly random samples of a homogeneous lot of seed. Where P is nearly equal to 1, $1 - P$ is very small, and the undue uniformity may have to be explained on grounds other than that of homogeneity of the sample. The only explanation that seems to fit such a situation is that the analyst, in an effort to achieve uniformity, has been unconsciously biased in his interpretations. This may result in an apparently satisfactory position of highly uniform results but the appearance is fictitious and can only be attained at the expense of uniformity between laboratories which it is the purpose of research in seed testing to promote.

In the paper by Porter (14) to which reference has been made previously the chi-square test was applied to the percentages of heavy and pure seed calculated from the analyses by the basic[3] method of 10 subsamples each from two lots of bluegrass. The probability values indicated that the differences were neither greater nor less than might be expected from homogeneous lots of seed. It appeared therefore that the particular blower unit with a constant speed motor and fan was operating uniformly. Furthermore, there was an indication that for lots with a purity of 72% the differences in replicates should not exceed 2.3 points. In contrast to these results 70 subsamples were drawn (15) from each of two lots of bluegrass and an attempt at analysis by the basic method was made using an improvised fan. The differences were considered extreme, and when the data were subjected to the chi-square test it was found that they were far greater than should be expected from homogeneous samples. To test further the constancy of air delivery by the new fan, 64 subsamples (16) were drawn from a lot of seed and analyzed by the basic method. The differences in purity percentages were found to be normal. Similarly, analyses of 32 subsamples each of orchard grass and red top seed by the basic method gave uniform results. A referee test (22) with 20 subsamples of reed canary grass seed analyzed by the basic method gave uniform results, whereas analyses of 20 subsamples from the same lot by 20 laboratories using the official method gave differences far greater than should be expected from a common population.

Porter further studied the germination of pure seed fractions obtained by employment of the basic method using bluegrass (15, 22), orchard grass and red top (22), and reed canary grass (22). When replicate fractions were tested under the same conditions in one laboratory the differences were found to be not significant.

The accuracy of separation by the Iowa separator was tested by repeated blowings of the same sample. The data obtained (16) showed that with 7 and 5 repetitions at the same valve opening the differences in extremes were 0.37 and 0.13, respectively, for lots with purity percentages of 69.37 and 88.69.

[3] In this discussion "basic" or "modified" method refers to the method on which the later refinements of the Iowa and Canadian methods are based.

A recent added feature to the Iowa Seed Separator is a vernier scale to the dial which makes it possible to adjust the valve opening to a fine degree of air flow. Operation of the separator can now be made with major dependence on the dial rather than on the manometer. It is frequently valuable, however, to be able to use both measurements and also to check one against the other. Pressure in the tube has been found to be more uniform and dependable than in the compression chamber.

In Canada, analyses made by the official method have been admittedly unsatisfactory. Leggatt has studied the problem from the viewpoint of developing a technique that would give statistically acceptable results.

In a recent visit to all the Canadian district laboratories he checked the calibration of the machines and conducted a series of tests on a single sample in order to determine the machine error, which was approximately 0.7%; that is, the highest and lowest tests on a single sample analyzed repeatedly, differed by this amount. The machine error was similar in the Ottawa type blowers installed in all laboratories except Toronto, and the Iowa type installed there. This error appears to be largely attributable to two causes, variations in moisture in the sample and variations in electric current. In the Ottawa type machines, the motor is 1/100 H.P. constant speed, 3,000 R.P.M. When it was suspected that such low power motors might be influenced sufficiently by current fluctuations to affect the results, even though slightly, a 1/12 H.P. motor was installed experimentally on the research laboratory machine. This has so improved performance that the only appreciable source of error now appears to be the variation in moisture. For example, the results of such a series of tests on a single sample are given in Figure 1. The day was bright and cold and the loss in weight is ascribable to moisture loss. Similar series of tests run on humid days exhibit an increase in total weight. Since it is the heavy portion which is subjected longest to the moving air column, that is the portion most affected by gain or loss of moisture. It will be seen that the total variation in this series of tests occurs during the first 11 tests and amounts to 0.4% and it is almost exactly paralleled by the moisture loss which amounts to 5 mgms.

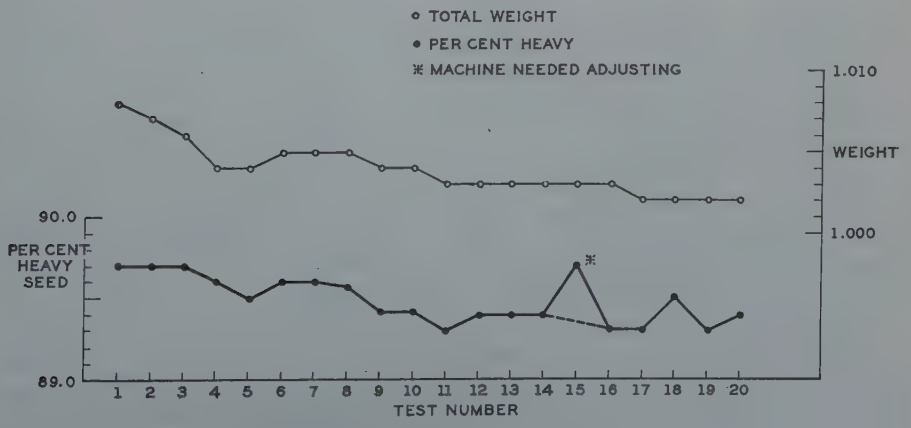

FIGURE 1.

In another series of similar tests carried out on a mild, moist day on a different sample (Sample G referred to later in connection with the discussion of the climax point as representing a type of sample presenting exceptional difficulty in analysis by the official method) the weight increased by 6 mgms. and the percentage by 0.6%, from 84.5% to 85.1%.

The actual machine error, therefore, appears to have been reduced to a minimum, and any further improvement in performance is not within the bounds of practical consideration depending, as it does, on the use of controlled humidity.

Such experiments revealed the capacity of the machine for accurate, repetitive work. The question he next considered was how this capacity is reflected in the analysis of different subsamples representing the same lot of seed. One series of tests may be reported here, on 16 one-gram subsamples of *Poa pratensis* representing a single lot of seed. They were prepared by dividing down 16 grams of seed by means of a sampler designed for fine seeds (10). The results are given in Table 1.

TABLE 1.—PERCENTAGES OF PURE SEED OBTAINED ON A SERIES OF 16 REPLICATES BY THE MODIFIED METHOD

Sample No.	Per cent pure seed
1	85.7
2	86.4
3	85.7
4	86.8
5	86.9
6	86.0
7	86.9
8	86.1
9	86.0
10	86.7
11	86.4
12	86.2
13	86.1
14	85.8
15	86.0
16	86.4
Mean	86.3
χ^2	10.596
P	.78

The value of P indicates that the only appreciable source of error is that due to random sampling. The fact that the P-value is rather high probably is ascribable to the effect of sampling from a restricted bulk which has been shown (11) to have a slight tendency towards a narrowing of the distribution curve.

In illustration of the increased uniformity attained in germination tests on pure seed samples resulting from successively heavier blowings, Table 2 is presented. These data, not previously published, are drawn from the same material as the "climax" blowing point paper (9).

TABLE 2.—DEVIATIONS PER CENT AND *P* VALUES OF 200-SEED DUPLICATE GERMINATION TESTS CONDUCTED ON PURE SEED FRACTIONS OBTAINED AT 11 SUCCESSIVELY HEAVIER BLOWINGS. DATA FROM 8 SAMPLES

Blowing	Average Deviation	*P*
1	7.64	<.01
2	8.50	<.01
3	6.72	.07
4	4.32	.20
5	3.18	.64
6 (climax point)	2.30	.96
7	2.22	.87
8	3.76 (3.28)*	.17 (.47)*
9	2.32	.53
10	3.38 (2.86)*	.05 (.36)*
11	1.82	.98

* Omitting one result which was markedly out of line.

The average deviation column makes a more immediate appeal because it is simply the averages of the percentage difference between each duplicate and its mate at each blowing. The values of *P*, however, give a truer picture of the uniformity because the germination tends to increase, the heavier the blowing, and the expected variability is in any case somewhat less for the higher germinations. The first column does not take this into account; the second does.

It will be noted that the variability between replicates decreases steadily to blowing No. 6 after which it remains low, if somewhat irregular. This decrease of variability is in part associated with the increase in percentage germination, as mentioned above, but the *P* values show that, apart from this, it is harder to secure satisfactory samples for germination from material which has been given lighter blowing. There were 14 pairs of replicates for comparison in blowings Nos. 4, 5, 6 and 7, but only 8 or sometimes 7 in the rest of the cases. No doubt the results would have shown greater regularity had more data been available.

THE NEW CONCEPT

The first published suggestion as to a need for a change in viewpoint concerning what constitutes pure seed was made by Porter in June 1938 (14) as follows: "Determination of absolute purity in small seeded grasses with multiple florets, such as bluegrass, is possible experimentally but is a practical impossibility and when applied by different seed laboratories results in variations much greater than may be expected from homogeneous subsamples of seed." "Practically it is less important to determine what is or is not a seed from the botanical standpoint than it is to determine the seeding value . . . . . any empirical method which gives a uniformly close approximation to the seeding value, measured in terms of purity and viability, should meet with some favour." In the same year this author prepared a paper for the Handbook of the Association of Official Seed Analysts (17) in which he called attention to the fact that pure seed is given in percentage by weight, and germination in percentage by number, and that the use of these two percentages in evaluating seed lots is accurate

only when the unit weights of viable and non-viable seeds in the pure seed fraction are approximately the same. This idea was further expressed in the Presidential Address (18) in August 1939, at Auburn, Ala., and a plea made for a new concept of pure seed. The author stated that "we must avoid retaining in the pure seed fraction those immature, non-fertile seeds or florets which cause a decrease in germination that is disproportionate to the increase in purity when such particles are retained as pure seed."

In order to confirm Porter's observations, using the Ottawa blower, Leggatt (who found himself fully in agreement with the former's general view) conducted in September 1939 a series of experiments (9) designed to determine the existence of a blowing point at which the utility value was at a maximum, which seemed theoretically necessary, arguing that at such point only was the true value of the number of pounds per 100 of viable seed to be determined. He proposed the term "climax point" to indicate the blower setting required to give the maximum utility value. For the climax point to be serviceable in practical seed analysis it is necessary that it should occur at or near the same setting for samples differing widely in quality, a question which was also studied at the same time. Using seven different samples varying from 17.5 to 25.7 lbs. per bushel and including one, Sample G (18.7 lbs.), in which occurred a large proportion of slim, partially developed, caryopses such as would afford the greatest difficulty in a test by the official method, he found that in all cases the climax point existed, that it was at a higher setting than that required to approximate the official test, and that it occurred at substantially the same point for all samples except sample G, for which it was slightly lower. It was found, however, that the percentage of heavy seed decreased slowly up to a point above the climax point after which it decreased sharply while the germination increased sharply up to a point below the climax point, after which it increased slowly. Between these two points on either side of the climax point is a region where decreases in heavy seed and increases in germination about cancel each other resulting in a utility value which remains substantially constant through this range. The range included sample G. It was concluded therefore that the climax point could be used as a standard point for widely different qualities of *Poa pratensis*, the species used in these tests, if taken on the low side of this range.

The advantages of this concept of pure seed may be summarized as follows. The botanical definition implicit in the official method is largely unworkable in practice owing to the impossibility of knowing for certain that a given "seed" conforms to such definition, particularly when viewed through investing glumes or in other cases to be discussed later. This lack of certainty has resulted in wide differences in interpretation, to the embarrassment of analysts and the disadvantage of the trade. According to the new concept, a strictly botanical definition is abandoned and in its place is substituted the idea of a mechanically determined dividing line between what shall be considered useful material and what worthless. This method results in a saving in time and in wear and tear on the laboratory personnel; in greater uniformity between analyses since interpretational error is no longer a factor; in less need for retesting because the heavier blowing produces a more uniform sample from which to draw the seeds used for germination; and, finally, the utility value derived by this

method more nearly reflects the actual number of pounds per hundred of strong, live seed than in the case of the official method.

It was the recognition of our virtual agreement upon the fundamental ideas involved that has led to the present joint statement and recommendation.

BASIC METHOD

The basic method by which the new concept of pure seed may be applied in practice has been developed primarily for small seeded grasses, principally Kentucky bluegrass seed. In principle it applies equally well to seed of orchard grass, reed canary grass, dallis grass, carpet grass, Bermuda grass, guinea grass, red top, Canada bluegrass, and chalcis-fly infested red clover and alfalfa. The method requires as standard equipment a vertical air blast separator equipped with a constant speed motor and fan, a manometer and a valve or gate with a fine adjustment for regulating the supply of air which passes through the seed. Such a separator must be capable of accurately separating two well defined fractions of a standard sample in a period of 5 minutes.

The Standard Sample

The standard sample is to be used solely for the purpose of preparing replicate standard samples.

It should consist of from 85 to 95% pure heavy seed of each grass species stained yellow and the balance of unfilled florets or spikelets stained red. The size of the standard sample and the proportions of pure heavy seed for the different species should be as follows:—

Species	Size of Sample	Pure heavy seed
Red top, *Agrostis alba*	0.5 ± 0.05 grams	90%
Ky. Bluegrass, *Poa pratensis*	1.0 ± 0.05 grams	85%
Can. Bluegrass, *Poa compressa*	1.0 ± 0.05 grams	85%
Carpet grass, *Axonopus compressus*	1.0 ± 0.05 grams	85%
Bermuda grass, *Cynodon dactylon*	1.0 ± 0.05 grams	95%
Reed Canary grass, *Phalaris arundinacea*	2.0 ± 0.05 grams	90%
Orchard grass, *Dactylis glomerata*	2.0 ± 0.05 grams	85%
Dallis grass, *Paspalum dilatatum*	2.0 ± 0.05 grams	85%
Guinea grass, *Panicum maximum*	2.0 ± 0.05 grams	85%

The relative weights of the pure and light fractions should be so adjusted that exact separation is obtained at the setting at which the separation of the given species is accomplished.

The setting at which the separation is accomplished is one already determined by the present authors. The Canadian proposal for bluegrass employs a setting at which an average sample of seed of *Poa pratensis* is separated in such a manner that the product of the pure, heavy seed and the percentage of germination is at a maximum. The Iowa method employs a setting which is used as the top pressure for a graduated scale ranging from a low to a high point. At the completion of the schedule at the high pressure the separation is practically the same as obtained in the Canadian method.

The standard sample must be prepared and preserved by a central laboratory equipped to prepare regularly replicate samples as required by other laboratories.

Replicate standard samples will be used to calibrate air blast separators so that a standard setting for each kind of seed may be determined and recorded. In general a valve or gate opening as well as a pressure reading should be recorded for each standard setting as determined by experiment.

Application of the Method to Bluegrass

The procedure followed in the analysis of Kentucky bluegrass seed by the basic method consists of 7 steps as follows:

1. Draw a sample of seed weighing 1 ± 0.05 grams from a bulk lot by means of a Boerner or other satisfactory divider.
2. Determine the weight per bushel of the one gram sample by the method and tables developed by Porter [15]. This step is not necessary if the bulk sample is of sufficient size to determine bushel weight with a laboratory scale.
3. Place in the cup of the separator and treat by one of the following schedules as preferred:

	Canada	Iowa
	Blow precisely 5 minutes at the standard setting.	Blow at 4 successive valve openings from low to high for 3, 3, 2 and 2 minutes, respectively, with the last opening at the standard setting. Empty the trap at the last two openings and save the material for separate examination.
4.	Examine the light fraction blown over for apparently viable seeds of other species and consider the balance as worthless material.	Examine the light fractions for apparently viable seeds of other species. Also examine the fractions removed at the last two openings separately with a 10X binocular and place normal, filled seeds in pure seed. Consider all the rest as inert.
5.	Remove from the heavy fraction all foreign seeds and all heavy extraneous material. All multiple florets are retained as pure seed.	Remove from the heavy fraction all foreign seeds, all heavy extraneous material and all completely empty multiple florets. Those with one good seed are retained. If the number is more than 50, separate and weigh, then add 1/3 to the inert and 2/3 to the heavy. This will be required only for certain samples below 21 lbs. per bushel although an occasional 21 lb. sample occurs that should be treated thus. After weighing place all heavy multiple florets with pure seed.

6. Count 4 × 100 particles for germination from the heavy fraction without regard to whether the caryopses are naked, or occur as single or multiple florets. Two or more sprouts from a multiple floret are counted as one.

7. Make all purity calculations on the basis of total final weight, not the original weight (2, 4, 18).

The differences in applying the basic method to bluegrass as outlined in the Canadian and Iowa procedures are not of major importance, they are minor in character and the results obtained by following either one should not be significantly different except possibly for low quality samples. The reason for the differences is that in Canada seed is marketed by grade and small differences in purity are not of importance. In the United States seed is sold on the basis of purity and germination. In general the trade places more emphasis on purity than on germination, and differences of 1 or 2% in purity of bluegrass are more difficult to adjust than differences of 5% in germination. Until the trade and seed analysts are educated to accept the new concept for pure seed in its entirety it is believed that the use of the Iowa procedure will be most acceptable in the United States.

Application to Other Species of Gramineae and to Leguminosae

The Iowa Method for orchard grass, several other grasses and chalcis-fly infested red clover and alfalfa seed is briefly as follows:

1. Orchard grass. (See references 16, 19, 25.)

Draw 2 ± 0.05 grams as described for bluegrass and blow at 4 successive valve openings from low to standard setting for 1, 1, 5 and 1 minutes respectively. Save the fraction removed at the standard setting separately and examine with a 10X binocular for pure seeds. Place florets removed in the proper category as described for bluegrass except that all heavy multiple florets are weighted together and 4/5 the weight added to pure seed and 1/5 to inert.

In the Canadian procedure the seed is sampled as above and blown at the appropriate setting as for bluegrass. Correction for multiple florets is made as above.

2. Dallis grass.

Draw 2 ± 0.05 grams and blow at 3 successive valve openings from low to standard setting for 2 minutes at each opening. Examine the material removed at the standard setting with a 10X binocular and remove any pure seeds to the heavy fraction. By pressing on the convex side of the spikelet with tweezers one can easily detect filled spikelets. Examine the heavy fraction with the binocular and remove all plainly diseased (including ergotized) spikelets. Because two or more spikelets frequently adhere it is often best to rub the heavy fraction lightly between the fingers and reblow 2 minutes at the standard setting. The heavy fraction after removal of diseased spikelets and other extraneous material is considered as pure seed.

3. Carpet grass.

Draw 1 ± 0.05 grams and blow at 3 successive valve openings from low to standard for 2 minutes at each opening. The fractions may be treated as described for Bermuda grass except that ergotized spikelets must be removed from the heavy fraction.

4. Bermuda grass.

Draw 1 ± 0.05 grams and blow 5 minutes at the standard setting. Treat the fractions as described for bluegrass (Canadian method) removing only seeds of other species and extraneous material from the heavy fraction.

5. Reed canary grass.

Draw 2 ± 0.05 grams and blow for 5 minutes at the standard setting. Treat the fractions as described for Bermuda grass.

6. Guinea grass.

Draw 2 ± 0.05 grams and treat as for reed canary grass using the proper standard setting.

7. Canada bluegrass.

Draw 1 ± 0.05 grams and treat by the Iowa Method or Canadian Method as described for Kentucky bluegrass except that the standard setting is slightly lower.

8. Red top.

Draw 0.5 ± 0.05 grams and blow 3 minutes at a setting below the standard and 2 minutes at the standard setting. Remove from the heavy fraction all extraneous material, diseased (including ergotized) florets, seeds of other species and consider the balance as pure seed. The small number of pure seeds in the light fraction may well be ignored or if preferred may be removed from that fraction removed at the top pressure.

9. Chalcis-fly infested red clover and alfalfa. (Iowa method) (5, 6, 23).

Draw a 5 gram sample and blow at 3 successive openings from low to standard for 3, 3 and 2 minutes respectively using 3 degrees as the interval on the dial.

In the Canadian procedure the sample is blown at the standard setting for 5 minutes.

Examine the portion removed at the maximum or standard opening for a few non-infested, normal or shrivelled pure seeds. If any are found, remove to pure seed fraction. Examine the heavy fraction for a few heavy but infested seeds which may be placed in the inert fraction. A binocular is of value for the examinations which may be accomplished in a few minutes. Infested seeds usually are mottled and without lustre. As experience is gained they are detected readily and are removed regardless of size. This method provides uniform pure seed fractions practically free from infested seeds. It will give a purity slightly lower than by the official method based on the slight change in the rules approved at the 1941 meeting of the Association, but the germination will be slightly higher, and if such seed is held for a few months the lower purity figure may be an advantage when the seed is tested later.

Possible Application to Other Seeds

Seed lots of endive, chicory, carrot, parsley, celery, many flowers, trees and shrubs frequently contain few to many undeveloped, immature seeds or fruits often with no embryo. Such particles are often not seeds or fruits even in the strictest botanical sense, yet they are included in the pure seed because it is difficult to classify them on the basis of the present definition of pure seed and because no practical method has been developed for classifying them.

Preliminary experiments indicate that the basic method described for grasses is equally applicable to other species. All that is needed appears to be a standard setting and the preparation and circulation of standard samples. Experiments in the Iowa Laboratory with carrot, celery, endive, chicory, dandelion, parsley and millets have given promising results. There is also good reason to believe that the method could be applied to seeds of small grains, rice, sorghums and many other field crop seeds.

THE BASIC METHOD IN PRACTICE

Porter has made extensive comparisons between series of analyses on uniform material by the Iowa method.

In May 1939 a set of 32 subsamples of bluegrass seed each weighing approximately one gram were drawn from a lot by the Iowa Laboratory and tested by the Iowa method. The percentage of heavy seed was recorded for each subsample, after which the fractions were carefully recombined. To each of 27 laboratories equipped with the Iowa Air Blast Seed Separator was sent one of the recombined subsamples. Each laboratory was requested to use the sample for calibrating its machine using the previously determined schedule as a guide. Thirteen of the laboratories returned the results which are shown in Table 3 together with the original percentage heavy as obtained by the Iowa Laboratory. In addition the percentage germination obtained by the Iowa Laboratory with 4 × 100 seeds of the heavy fraction returned by each co-operator is given.

Table 3.—Separation of replicate samples of bluegrass by the control separator (Iowa) and similar separators in other laboratories, 1939

Laboratory Number	Purity Percentage		Germination percentage Iowa Lab. from pure seed fraction sent by Co-operating Laboratory
	Iowa	Co-operating Laboratory	
1	90.2	90.1	85.0
2	90.3	89.9	85.3
3	89.5	89.3	82.0
4	90.0	90.0	88.0
5	89.5	89.7	86.0
6	90.0	90.3	83.0
7	90.0	90.1	84.8
8	89.8	90.1	86.3
9	89.8	89.7	84.0
10	90.4	90.3	87.3
11	89.9	89.6	84.8
12	90.2	90.3	83.0
13	90.4	90.8	84.5
Mean	—	—	84.9
P	—	—	.50

The data in Table 3 illustrate two points: (1) calibration of the machines was uniformly satisfactory, and (2) the pure seed fractions gave reasonably uniform germinations under the same conditions.

In May 1940, 32 sets of subsamples each from 2 lots of bluegrass, one lot of orchard grass and one lot of reed canary grass were drawn in the Iowa Laboratory and each was analyzed by the basic method of procedure. Each of 26 laboratories equipped with an Iowa Air Blast Separator received one subsample from each of the four lots. Each of 13 laboratories analyzed the subsamples of bluegrass by the basic method without knowledge as to the result obtained by the laboratory with the control machine. The results given in Table 4 show the percentage purity obtained by each laboratory in comparison with the result in the Iowa Laboratory. In only two cases out of 13 each for lots A and B are the differences between the percentages from identical samples of any importance, and in those cases the differences are small. In general the uniformity is quite satisfactory and certainly far more so than has ever been obtained by the use of the official method.

TABLE 4.—ANALYSES OF REPLICATE SAMPLES OF GRASSES BY A CONTROL AND CO-OPERATING LABORATORIES USING THE BASIC METHOD

Laboratory No.	Bluegrass				Reed Canary		Orchard grass	
	A		B		Control	Co-op.	Control	Co-op.
	Control	Co-op.	Control	Co-op.				
1	82.8	82.9	89.5	89.8	93.5	93.7	86.8	87.4
2	82.6	82.3	90.0	90.3	92.7	93.0	87.1	86.4
3	83.2	84.4	89.7	90.0	92.5	92.6	85.8	86.4
4	83.3	83.8	88.8	88.0	92.2	91.9	86.0	87.6
5	82.9	82.9	89.3	89.0	92.8	93.1	85.0	85.2
6	82.4	83.9	89.3	89.3	93.7	93.6	—	—
7	82.2	82.6	89.7	89.7	92.2	90.3	87.3	87.4
8	83.1	83.2	89.3	88.6	92.1	92.2	86.4	86.6
9	83.2	83.3	89.6	89.0	92.5	92.8	85.1	85.3
10	83.7	84.5	89.1	89.8	92.6	93.0	86.4	86.6
11	82.8	82.0	89.7	89.2	93.3	93.7	85.5	86.1
12	81.9	81.9	89.1	90.1	—	—	—	—
13	82.3	81.4	89.5	87.7	92.8	92.9	87.0	87.4

The subsamples of reed canary grass and orchard grass were sent for the purpose of calibration of the seed separators. The percentage purity obtained in the laboratory with the control machine was supplied with each subsample as a guide. The results with reed canary grass are remarkably similar in each comparison except for No. 7. The data obtained with orchard grass are of particular significance because the method required separation by the air blast into heavy and light, then classification of the heavy fraction into single and multiple florets and calculation of 4/5 the weight of heavy multiple florets for inclusion as pure seed. With the

exception of laboratory No. 4 the uniformity obtained with identical samples is truly remarkable, especially since it was the first attempt to standardize the method for orchard grass by use of identical subsamples.

A third example of the basic method in practice is given in Table 5 which shows the results of purity tests made on 13 lots of bluegrass seed in the Iowa Laboratory in comparison with the results obtained by the seed company that submitted the samples. The sample of each lot was sent to the Iowa Laboratory as a routine procedure in the regular preparation of the lot for marketing. Laboratory samples were also drawn for use by the company analyst who had been using an Iowa Air Blast Separator for more than one year. In no case are the differences between the two laboratories significant, yet the tests were made without previous knowledge by either analyst of the other's results. The data in Table 5 not only indicate uniform analytical procedures but good bulking and sampling methods. This particular seed company uses the seed separator to check bulking and mixing of lots before preparation for shipment.

TABLE 5.—PURITY ANALYSIS OF 13 LOTS OF BLUEGRASS BY TWO SEED LABORATORIES USING THE BASIC METHOD

Lot	Percentage pure seed, bluegrass		Difference
	Iowa	Company	
1	78.02	77.96	0.06
2	82.40	83.07	0.67
3	85.58	85.15	0.43
4	80.77 } Av. = 81.53	81.09 { Lots 4 and 5 were combined before analysis }	0.44
5	82.30 }		
6	81.90	81.71	0.19
7	85.26	84.24	1.02
8	80.30	81.33	1.03
9	94.63	94.71	0.08
10	96.63	96.32	0.31
11	90.76	90.05	0.71
12	86.29	85.35	0.94
13 Can. Bluegrass	87.14	87.17	0.03

A fourth illustration is given in Table 6 which shows the results of purity tests on 12 lots of Kentucky bluegrass seed, samples from which were sent by the processor to two state laboratories each equipped with the Iowa Air Blast Seed Separator. The uniformity achieved is remarkable and has never been attainable by the use of the official method.

TABLE 6.—PURITY ANALYSIS OF 12 LOTS OF BLUEGRASS BY TWO SEED LABORATORIES USING THE BASIC METHOD*

Lot	Percentage pure seed, bluegrass		Difference
	Kentucky	Iowa	
1	82.40	81.86	0.54
2	76.27	76.82	0.55
3	87.05	86.69	0.36
4	98.27	97.87	0.40
5	88.47	86.56	1.89
6	85.18	86.11	0.93
7	85.95	88.05	2.10
8	87.09	87.68	0.59
9	98.09	97.91	0.18
10	67.89	68.02	0.13
11	89.44	87.92	1.52
12	85.10	84.59	0.51
Mean	85.93	85.85	

* Published with approval of Kentucky Agricultural Experiment Station.

A fifth illustration of the basic method in practice is given in Table 7 which shows the results of purity analyses by 9 laboratories using the basic method with two low quality samples containing many multiple florets. The samples were prepared in the control laboratory, not previously analyzed but submitted as regular routine samples. The differences are significant but considerably less than commonly experienced when the official method is employed. No correction for multiple florets was used in these tests. It is probable that further calibration and standardization will reduce these differences with low quality lots. For lot A the differences are less than allowed in the tolerances under the Federal Seed Act and for lot B they are the same.

TABLE 7.—ANALYSIS OF TWO LOW QUALITY LOTS OF BLUEGRASS BY 9 LABORATORIES USING THE BASIC METHOD

Laboratory No.	Percentage pure seed	
	Lot A	Lot B
1	56.8	78.3
2	55.6	74.9
3	57.4	74.1
4	54.2	74.8
5	57.1	76.7
6	55.9	78.7
7	52.6	73.4
8	58.1	77.7
9 Control	53.5	73.5
Mean	55.7	75.8
χ^2	52.8	86.4
P	< .01	< .01
Diff. in extremes	5.5	5.3
Tolerance F.S.A.	7.97	5.29

Leggatt, having established the fact that the modified method accurately reflects the sampling variation in replicate samples using the research laboratory machine, designed the following experiment to determine how nearly this was the case when replicate samples were analyzed by the different district laboratories.

Nine subsamples were taken at random from 24 subsamples previously drawn from 24 grams of seed. One was sent to each laboratory and one retained by the research laboratory. Each laboratory was asked to blow their sample by the uniform method and to remove all extraneous material from the heavy portion. The three separations, heavy pure seed, heavy extraneous material and the light fraction were kept separate and returned to the research laboratory where the pure and light fractions were remixed and blown and the heavy extraneous fraction weighed. The same procedure was followed with the sample retained by the research laboratory. The results obtained are given in Table 8.

TABLE 8.—REPLICATE SUBSAMPLES ANALYZED BY DIFFERENT STATIONS AND RE-ANALYZED BY SEED RESEARCH LABORATORY

Station	Percentage pure seed reported by		Difference
	Station	Seed research	
	%	%	
1	84.6	84.9	− 0.3
2	83.5	84.2	− 0.7
3	85.9	86.3	− 0.4
4	84.4	85.2	− 0.8
5	85.7	85.5	+ 0.2
6	86.5	85.9	+ 0.6
7	85.2	85.0	+ 0.2
8	85.4	84.7	+ 0.7
Seed Research	85.2	85.1	+ 0.1
Mean	85.16	85.20	
χ^2	25 approx.	12.615	
P	< .01	.15	

The magnitude of χ^2 obviously is affected by the fact that one sample, that received by station No. 2 is out of line, a fact which is confirmed by its being so in both series of results. That is, its low value is not due to error of the machine. The value of P is $< .01$ for the series done at the different laboratories and .15 for the series done at the research laboratory, both of which are low values. If the sample in question is omitted and χ^2 recalculated, losing one degree of freedom, P is .07 for the first series and .32 for the second. Thus the second series can be considered entirely satisfactory and the first, reasonably so. That the calibration of certain of the machines requires checking is suggested by the "difference" column.

It is a fact of common experience in the Canadian laboratories that this is a much higher degree of uniformity than was ever attained by the official method.

DISCUSSION

Since the development of seed testing, the establishment of local and national laboratories on the North American Continent, in Europe and in other parts of the world and organization of two seed testing Associations

one of the most persistent and perplexing problems has been non-uniform results of purity analyses as obtained by two or more laboratories with samples of seed drawn from the same lot. Records of referee tests by the Association of Official Seed Analysts and by the International Seed Testing Association clearly show differences greater than should be expected with homogeneous samples of seed. These differences have been particularly marked when small seeded grasses (bluegrass and orchard grass) were involved. Published reports of referee tests by the Research Committee of the Association of Official Seed Analysts between 1937 and 1941 are of some significance.

Carefully conducted tests by Porter (15, 16, 18) and by Leggatt (4, 8) clearly show that when a carefully controlled procedure such as is described in this paper is followed with certain grasses, the percentages of purity obtained have been remarkably uniform and within a normal range of variation. Further, the pure seed fractions obtained have been uniform as shown by germination tests. Also it has been shown that when other laboratories are equipped with similar apparatus it is possible to obtain results of almost surprising uniformity.

It appears, therefore, that efforts to apply the existing official definition of pure seed as recommended by the Association of Official Seed Analysts to the practice of seed testing have not achieved the uniformity warranted by the material. The reason for this failure is obvious. It is inherent in the character of the material involved, in the chance for too much personal interpretation, and in the faulty operation or design of old type seed separators.

A second drawback to the application of the official definition of pure seed is that the purity percentages obtained when used in conjunction with percentage germination have not given a correct measure of seeding value. One of the major functions of seed testing is to provide a measure of the value of a given lot of seed. A reasonable measuring stick is that of pounds per hundred of pure viable seed. Musil (13) proposed the direct method which may well be the most accurate measure of seed value in that it gives the number of viable seeds per unit weight. It might be difficult to apply this method to seed labelling. The commonly accepted procedure is to determine percentages of purity and germination, multiply the two and divide by 100. The resultant figure is considered to represent the number of pounds per hundred of pure viable seed.

Porter (17) has pointed out that since purity is determined by weight and germination by number the index figure is incorrect unless the unit weights of the viable and non-viable seeds in the pure seed fraction are the same. In most grasses the more immature and light weight the seeds are the lower is their vitality. This means that as the percentage of such seeds increases in the pure seed fraction the percentage germination of the pure seed is decreased disproportionately to their proportion by weight. For example, let us assume that a sample of bluegrass with a purity of 85% by the official method has a pure seed fraction composed of 95% by weight of heavy plump seeds, the balance of 5% consisting of variable seeds ranging from poorly developed to fairly mature. Assume further that 7 of the seeds in the 5% portion are equal in weight to 5 of the heavy plump

seeds (a sound assumption) and that 5 of the heavy plump seeds weigh approximately 1 mg. Starting with a 1-gram sample there would be 850 mg. of pure seed consisting of $807.5 \times 5 = 4{,}037.5$ seeds, and in the second $42.5 \times 7 = 297.5$ or a total of 4,335 seeds in the pure seed fraction. The ratio by number of the two classes is 13.57 to 1.

Let us assume further that the percentage germination of the heavy seeds is 90 and that the second fraction of pure seeds is divided evenly by weight into two sub-groups, Group 1 with a germination of 60%, Group 2 with 20% and a weighted mean of 37% for the second fraction as a whole, which means that Group 1 is 42.5% of the second fraction by number and Group 2, 57.5%. Using the pure seed fraction obtained by an official purity test from which to draw seeds for germination, the calculated percentage germination would be $\frac{4{,}037.5 \times 0.90 + 297.5 \times 0.37}{4{,}335} = 86.36$. The index value would be $\frac{85 \times 86.36}{100} = 73.4$. Inasmuch as the ratio of heavy to light weight seeds in the pure seed fraction by weight is actually 19 to 1 instead of 13.57 to 1, the real measure of germination should be $95 \times 0.90 + 5 \times 0.37 = 87.35$. The index value should therefore be $\frac{85 \times 87.35}{100} = 74.25$. The difference of 0.85 in the index values by the two calculations is of some importance and the value of 74.25 undoubtedly more correctly represents the number of pounds per hundred of pure live seed.

Using the basic method (Canadian procedure) which employs the "Climax Point," the assumption is that the standard setting would be so adjusted that half the lighter fraction of the pure seed by weight would be removed which would reduce the purity to 82.875%. The germination would therefore be $\frac{4{,}037.5 \times 0.90 + 126.5 \times 0.6}{4{,}164} = 89.09$ and the index value would be $\frac{82.875 \times 89.09}{100} = 73.83$.

Using the basic method (Iowa procedure) slightly more of the lighter fraction would be retained in the pure seed than by the Canadian and the germination might be slightly reduced. It is estimated that the purity would be 83.3 and the germination 89. The index value would therefore be $\frac{83.3 \times 89}{100} = 74.14$ which is close to the actual value of 74.25.

It should be pointed out that the index value obtained by employing the official method of purity analysis is an extremely variable factor for any one lot of seed because of the wide difference in personal interpretation as to what constitutes pure seed as well as to differences resulting from variable methods and interpretation in germination tests. Reference to the paper by Porter and Brown (1) and to referee tests by the Research Committee of the Association of Seed Analysts (21, 22) will verify the truth of this contention. The index value may range from low to high

depending upon how nearly the procedure approaches the basic method herein outlined. The data that have been accumulated by the authors justify the conclusion that the basic method gives far more uniform pure seed fractions and index values than does the official method. This point is illustrated by the data in Table 9 adapted from Table 19, page 46 of the Proceedings of the Association of Official Seed Analysts in 1938. Column 1 shows the percentages of pure seed obtained by 26 laboratories with subsamples from a given lot of seed. The differences are highly significant, the percentages ranging from 87.4 to 94.2. Actually the subsamples were from a homogeneous population as shown by previous analysis of each sample by the basic method and illustrated in column 4 of the table. The

TABLE 9.—COMPARISON OF INDEX VALUES OBTAINED BY 26 LABORATORIES WITH PREVIOUSLY DETERMINED HOMOGENEOUS SUBSAMPLES OF BLUEGRASS SEED*

Laboratory purity Official method	Laboratory germination	Index value $\frac{P \times G}{100}$	Iowa method purity
89.4	85	75.99	88.3
90.6	84	76.10	89.1
88.9	87	77.34	88.6
94.2	55	51.81	89.0
90.2	82	73.96	89.6
90.4	88	79.55	88.5
90.2	82	73.96	88.4
92.4	79	73.00	88.6
91.2	85	77.52	88.0
91.0	76	69.16	88.7
91.0	69	62.79	88.5
93.8	85	79.73	89.4
91.4	79	72.20	88.6
89.2	79	70.47	89.3
91.6	83	76.03	88.9
89.5	84	75.18	88.4
92.6	78	72.23	89.1
90.6	80	72.48	88.9
88.0	83	73.04	89.4
91.6	84	76.94	89.0
92.1	81	74.60	88.8
88.4	88	77.79	88.1
91.1	81	73.79	87.8
91.2	87	79.34	88.2
90.3	91	82.17	89.9
87.4	86	75.16	88.6

* Mean purity of this lot determined from 64 subsamples was 88.64 per cent. Germination was 88.5

Index value $\frac{88.64 \times 88.5}{100} = 78.45.$

germination percentages and index values for each laboratory are given in columns 2 and 3 of the table. Variations in germination are in part caused by significant differences in pure seed fractions and in part by different germination techniques. The range in index values is from 51.81 to 82.15. Tests of 64 subsamples from this lot by the basic method in the Iowa laboratory gave a mean purity percentage of 88.64 and repeated germination tests with 4 × 100 seeds from the uniform pure seed fractions gave a mean germination of 88.5%. The index value therefore may be considered as

about $\frac{88.64 \times 88.5}{100} = 78.45.$

The most accurate method of determining index value of bluegrass when purity and germination are considered is to remove all the light weight shrivelled seeds by blowing, place the inert fraction in the germinator and count the number of normal sprouts obtained. Allowing a value of 7 sprouts in the inert as equal in weight to 5 normal seeds would mean that for every 7 sprouts (equal to 1 mg.) a correction of 0.1% should be made in the pure seed percentage or more correctly 1/10 point added to the index value because the germination of such sprouts is 100. In the theoretical problem if all the lighter fraction were removed and placed in the germinator the calculation would be $\frac{80.75 \times 0.90}{100} = 72.68$ the index value for the heavy seed. For the lighter fraction it would be $\frac{297.5 \times 0.37}{7 \times 10} = 1.57$. The actual index value of the sample in question would be $72.68 + 1.57 = 74.25$ which is the same as calculated when the ratio of 19 to 1 for heavy and light in the pure seed fraction was used. There is no practical way to compute this value however except by germination of the inert fraction.

The Iowa method as first developed provided for the correction as measured by the number of sprouts in the inert fraction, with the correction made in pure seed rather than in index number. It was later modified to an examination of the fractions removed at the two higher pressures and a removal of small but firm seeds to the pure seed fraction. The results by the two procedures are similar and should provide an index value slightly above the Canadian or "Climax Point" method. On the other hand the sprouts produced by the inert fraction or by the small seeds recovered by hand are usually less vigorous and less normal than those from heavy seed hence for all practical purposes the advantage gained is of little consequence and allows for a personal interpretational error. The only advantage of the Iowa procedure is that it gives a slightly higher purity for some samples, particularly those of medium to low purity. If less emphasis were placed on high purity by the seed trade, the Canadian procedure would be preferable.

It is interesting in this connection to compare the S. M. as accepted and used by European laboratories with the official method of the Association of Official Seed Analysts and the proposed method based on the new concept of pure seed. The S. M. may be considered more comparable to the proposed new method in that undeveloped seeds of low value are removed from the pure seed by both methods. It seems probable that the proposed method would be superior to and more uniform than the S. M. because of (1) saving in time and (2) little chance for personal interpretational errors. The adoption of the proposed method would help to bring the two seed testing associations into closer agreement. Differences of opinion on this major point of purity determination have prevented full co-operation and understanding between the two associations.

There is little doubt that the present official method gives an index value considerably below the actual value for certain lots of vegetable and flower seeds which contain many shrivelled and undeveloped seeds with

little or no vitality. The same is true for many samples of chaffy grasses and for clover seed heavily infested by chalcis-fly. It also requires much time with oats. Samples with many broken seeds are likewise undervalued. The proposed change would not only give a more correct value to many lots of seed but would largely eliminate one of the most important sources of non-uniform tests which have long been characteristic of seed testing methods. Two quotations from the paper by Porter (15) in 1938 are significant in this connection. He stated "The adoption of the modified method of analyzing bluegrass for purity would involve the following:

(*a*) Installation of a standard control machine in a laboratory where frequent checks on samples could be made for all laboratories with similar equipment.

(*b*) Purchase of a scale and use of graduated vials for determining weight per bushel of small samples.

(*c*) Acceptance of the principle of approximate purity by a uniform method of procedure as a substitute for the ideal of absolute purity.

(*d*) Regular examination of pure seed remnants with a binocular or reflected light to check the reliability of the fan."

"The use of a standard method of procedure based on the principle of separation of fertile and infertile florets by air pressure, as developed and maintained by a uniform speed motor, would solve one of the most difficult labelling and analytical problems now faced by the seed trade and seed analysts, respectively, and in addition provide uniform pure seed fractions for germination which should automatically reduce some of the differences obtained in germination tests."

A further advantage to the adoption of the basic method is that when germination tests only are requested the fractions prepared for germination could be the same as if a purity test were made, thus providing an additional uniform procedure.

CONCLUSION

The authors recommend to the Association of Official Seed Analysts and to the International Seed Testing Association that serious consideration be given to this new concept of pure seed and to the proposed methods of applying it in routine laboratory analyses with a view to accepting it as a basic and fundamental procedure in seed technology. Similarly the authors respectfully urge the American Seed Trade Association to weigh carefully the advantages to seed merchandising which this proposed new concept applied to seed testing should provide. Less difficulty would be incurred in marketing, and more emphasis would be placed on cleaning of low grade samples in order to raise the purity and the viability simultaneously.

Finally, the proposal should receive serious consideration by government officials who regulate the importation of seed stocks from foreign countries. At present importations are governed by a minimum pure live seed content. The proposed method based on the new concept of pure seed would give a more accurate measure of pure live seed and thus be fairer to seed importers.

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THE INHERITANCE OF FLEECE WEIGHTS IN RANGE SHEEP[1]

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INTRODUCTION

Wool production is one of the triple functions of sheep from the standpoint of their economic value in animal husbandry. In the range areas of North America wool and meat are the two commercially important products. The relative returns from wool and meat fluctuate with the selling prices of the two products and also with the amount of each produced per breeding unit in the flock.

Increase in wool production per unit may result from improvement in environmental conditions, improvement in the hereditary productive ability, or from both causes. Some studies have been made of the inheritance of several fleece characteristics, but relatively little attention has been given to fleece weight. Yet fleece weight affects the income of the sheep breeder more than other fleece characteristics do. The present study was undertaken to learn more about the inheritance of fleece weights and how to make more rapid improvement in this characteristic.

REVIEW OF LITERATURE

Repeatability of fleece weights has been studied by several investigators with the hope of learning more definitely the relation between the weights of successive fleeces from the same sheep. Hill (5) and Lush and Jones (12) found correlations of about 0.60 between successive fleeces. Gartner and von Ungern Sternberg (4) obtained a correlation of 0.61 ± 0.04 between yearling fleece weight and life wool yield and Terrill (17) reported a correlation of 0.59 between yearling fleece weight and lifetime yield (2 to 5 years). Vaughan, Joseph, and Vinke (20) and Joseph (8) reported correlations ranging from 0.29 to 0.84 between fleeces from sheep of various ages. Thomson (19) obtained values of 0.72 for sheep in Alberta and Johannson and Berg (7) found the average intra-herd correlation for fleece weight to be about 0.35 for various breeds in Sweden.

Only a few daughter-dam correlations and regressions of fleece weight have been reported. Phillips *et al.* (14) studied these statistics for unscoured fleece weights of 649 pairs of Corriedales and 639 pairs of Rambouillets. The correlation coefficients ranged from 0.03 to 0.51 for the Corriedales and from 0.01 to 0.40 for the Rambouillets with corresponding mean values (calculated by Fisher's z method) of 0.30 and 0.17. The mean values of the regression coefficients were 0.30 and 0.19 respectively when each regression was weighted by the number of degrees of freedom associated with it. Briggs (1) obtained a correlation of 0.58 between fleece weights of daughters and dams from a small group of Rambouillet sheep.

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SOURCE OF DATA

The data utilized in this study comprised the shearling fleece weights from two flocks. The records for the Canadian Corriedales and the Rambouillets came from the flock at the Dominion Experimental Station, Lethbridge, Alberta. Those for Romney crossbreds were from the Dominion Range Experiment Station, Manyberries, Alberta. The data for the Canadian Corriedales were from sheep born in the period 1930 to 1939, for the Rambouillets 1933 to 1939, and for the Romney crossbreds 1936 to 1939 inclusive.

The Canadian Corriedales, so named to distinguish them from New Zealand Corriedales, have been developed at the Lethbridge Station from an original cross of a Lincoln ram on selected Rambouillet ewes in 1919. Female progeny from this cross were graded to New Zealand Corriedale rams until 1934 since which time inbreeding within several lines has been followed.

The Rambouillet flock for the first few years was small but in 1938 it was augmented by the purchase of 125 yearling ewes and 75 mature ewes from four breeders in Montana and Utah. Only the yearling and their progeny were included in this study.

The Romney crossbreds at the Dominion Range Station originated from a cross of 12 Romney rams, purchased from a breeder in Oregon, on selected grade Rambouillet ewes in 1935. The F_1 progeny from this cross were interbred, without close inbreeding, to produce the F_2 generation. Only F_1 and F_2 data were available for this study.

The sheep at the Lethbridge Station have been on range in the mountains during the summer months except in 1935 and 1936 when they were on prairie range. During the fall and winter months they have been kept at the Station which is located in an irrigated area. In open weather pasture has been provided by grain and alfalfa stubble. When the weather prevented winter grazing and during the breeding season, when the sheep were in the breeding pens, alfalfa hay and some grain was fed.

At the Range Station the sheep have been maintained under range conditions such as exist in the drier, short-grass plains area of Western Canada. Very little winter feeding has been done except for the ewe lambs. These have at times been wintered on stubble fields and given supplemental feed.

Shearing was done usually about the second week in May at the Lethbridge Station and the first week in June at the Range Station. The fleece weights were taken immediately after the fleeces were removed from the sheep and had been rolled for packing. The weights were taken to the nearest tenth of a pound on spring scales of good quality. No skirting or sorting was done but heavy dung locks were removed if they were present.

Lambing started about the first of April at the Lethbridge Station and about one month later at the Range Station. The lambing season each year extended over a period of about five weeks. Differences of that magnitude in the age of the sheep at the time of first shearing might have a noticeable effect on fleece weight and then would require some correction.

However, a study of the present data showed no consistent or simple effect of these differences in age, possibly because environmental conditions, as much as age *per se*, could cause differences between lambs born at different periods of the lambing season. A different correction would have been required for each year so none was made.

ANALYSIS OF DATA

General Considerations

Correlations, regressions, and analysis of variance were the statistical tools employed in analyzing the available data. These procedures are quite standardized and require no general discussion in this paper. Their application will be discussed with each phase of the analysis.

If the data could have been analyzed on a clean wool basis, some of the variation caused by differences in the moisture, suint, and dirt content of the fleeces could have been eliminated. This was not possible for the data included in the present study. Furthermore, in the selection of sheep, unscoured fleece weights must be used. Consequently, for practical reasons it is important to know about differences in the weight of the unscoured fleeces. Fortunately unscoured fleece weight and yield of clean wool are highly correlated in populations of unselected fleeces. Spencer *et al.* (16) found the correlation to be 0.62 ± 0.01 for 990 Rambouillet fleeces. Malan *et al.* (13) reported the correlation to be 0.67, 0.80, 0.79, for three separate years. Terrill (18) obtained a correlation of 0.69 for 186 yearling fleeces from Rambouillet sheep.

General Statistics of the Populations Used in This Study

Table 1 presents a general summary of the means and standard deviations of the weights of shearling fleeces produced by the three flocks under study.

TABLE 1.—STATISTICS OF SHEARLING FLEECE WEIGHTS FOR THE THREE BREED GROUPS

Year of birth	Canadian Corriedale			Rambouillet			Romney Crossbred		
	n	Mean fleece wt.	σ	*n*	Mean fleece wt.	σ	*n*	Mean fleece wt.	σ
1930	15	9.00	1.07	—	—	—	—	—	—
1931	20	8.62	0.98	—	—	—	—	—	—
1932	26	9.66	2.09	—	—	—	—	—	—
1933	29	10.21	1.25	7	8.67	1.60	—	—	—
1934	33	9.67	1.19	6	9.20	1.21	—	—	—
1935	42	8.23	1.24	8	7.70	1.73	—	—	—
1936	45	7.62	1.10	3	6.70	1.15	192	6.28	1.01
1937	26	9.35	1.79	11	9.13	0.73	202	7.58	1.10
1938	52	7.51	1.14	52	7.98	1.31	67	8.79	1.35
1939	44	8.15	1.01	43	7.43	1.12	146	6.87	1.01
Totals	332			130			607		
Averages		8.60	1.30		7.94	1.25		7.13	1.10

NOTE.—Throughout this paper the year designates year of birth but fleece weight data are for the following season.

The difference between the Corriedale and Rambouillet means is significant and constitutes a real breed difference. The mean of the Romney crossbreds also differs significantly from the other two but, since the Romneys were raised under different conditions, it is not clear whether this is a breed difference or an environmental difference.

The standard deviations of shearling fleece weights in these data are lower than those obtained by Phillips *et al.* (14) for somewhat similar data. However, their mean fleece weights were higher and when coefficients of variation were calculated for their data it was found that these were slightly lower (13.7, 14.6, and 14.6 for Columbias, Corriedales, and Rambouillets, respectively) than those obtained for the present data namely, 15.1, 15.7, and 15.4 for Canadian Corriedales, Rambouillets, and Romney crossbreds.

Repeatability of Fleece Weights

Repeatability of fleece weights was studied to determine outside limits of heritability and to learn whether the flocks in this study were comparable to others that have been studied. This is the only part of the present study in which fleeces from sheep older than shearlings were included. All sheep that had recorded three fleeces in their first three years of life were included in this analysis. In this manner data were obtained for 196 Corriedales, 81 Rambouillets, and 282 Romney crossbreds. The analyses were made separately for each of several years and then were combined to provide for each breed a pooled estimate of the intra-year, intra-class correlation. The results are shown in Table 2.

TABLE 2.—INTRA-YEAR, INTRA-CLASS CORRELATIONS BETWEEN WEIGHTS OF FLEECES FROM THE SAME SHEEP

Year of birth	Canadian Corriedales		Rambouillets		Romney Crossbreds	
	Number in group	Intra-class correlation	Number in group	Intra-class correlation	Number in group	Intra-class correlation
1930	11	0.24	—	—	—	—
1931	15	0.35	—	—	—	—
1932	22	0.44	—	—	—	—
1933	29	0.53	6	0.31	—	—
1934	26	0.57	—	—	—	—
1935	29	0.54	15	0.72	—	—
1936	39	0.63	40	0.52	166	0.41
1937	25	0.79	20	0.63	116	0.46
Total number	196		81		282	
Average correlation		0.56		0.56		0.43

These intra-class correlations were highly significant statistically and their numerical value is of much the same order as correlation coefficients obtained by other workers who have investigated this subject. It appears that roughly half (a little more in the Corriedales and Rambouillets, a little less in the Romneys) of the differences between weights of unscoured

fleeces from one sheep to another at a given shearing are caused by permanent differences between those sheep and will be found again in future shearings.

The difference between the Corriedales and the Romney crossbreds was significant at the 5% level but the difference between the Rambouillets and the Romney crossbreds was not significant. The lack of significance in this latter case, despite the similarity of the correlations for the Corriedales and Ramoubillets, was caused by the smaller number of animals in the Rambouillet group.

Daughter-Dam Correlations and Regressions

One method of obtaining an estimate of the heritability of a characteristic is through studying the likeness between parent and offspring. Both correlation and regression of offspring on dams were used to measure the parent-offspring resemblance. The regression offered some advantage in avoiding bias that might have been introduced by selection of the dams.

In calculating the daughter-dam correlation and the regression of daughter fleece weight on the fleece weight of the dam the adjustment for environmental influences was accomplished by grouping the pairs of daughters and dams on a double intra-year basis. That is, all daughters born in one year were placed in sub-groups with dams all born in a single year. Thus the sheep in each daughter sub-group would have been subject to the same general environmental influences applying to the flock that year and the members of each sub-group of dams would all have been under similar general environmental influences. Differences between contemporary individuals then would be caused by genetic differences and by the environmental factors peculiar to each individual. Environmental factors common to animals in each sub-group of dams and in each sub-group of daughters would cancel out.

Grouping on this basis led to 31 sub-groups for the Canadian Corriedales, 13 sub-groups for the Rambouillets, and 3 sub-groups for the Romney crossbreds. The variance and the co-variance was calculated separately for each sub-group. These were then pooled to provide an estimate of the average correlation and regression coefficients for each breed by the method outlined by Snedecor (15). The coefficients thus obtained were on an intra-year basis; i.e., concerned deviations from the average of contemporaries in the same flock. The results are shown in Table 3.

TABLE 3.—AVERAGE INTRA-YEAR DAUGHTER-DAM CORRELATIONS AND REGRESSIONS FOR THE VARIOUS GROUPS OF SHEEP

	Corriedales, pedigree group	Rambouillets	Romney cross-breds	Corriedales, all groups
No. of daughter-dam pairs	173	70	213	206
Intra-year correlation	0.16	0.19	0.05	0.22
Intra-year, intra-sire correlation	0.14	0.22	0.07	
Intra-year, intra-sire regression	0.12	0.20	0.07	

In the Corriedale data the daughters born in the first three years were by New Zealand Corriedale sires and those in the last five years were by Canadian Corriedale sires. The daughters of the New Zealand Corriedale sires were from flock matings so that the sires for individual daughters could not be identified. Daughters by Canadian Corriedale sires were from pen matings so both the sire and dam were known. Therefore, the main discussion in this paper will be limited to the 173 daughter-dam pairs in which the daughters were sired by Canadian Corriedale sires. The correlation for all the Corriedales was 0.22 whereas for the pedigreed group the correlation coefficient was 0.16.

None of the correlation coefficients are statistically significant; hence it cannot be determined whether the observed differences have any significance. However, the small size of the correlation for the Romney crossbreds suggests that it may be different from the other two.

The elimination of sire effect did not markedly change the numerical values of the correlation coefficients. In the Corriedale group the coefficient was reduced slightly, whereas in the other two groups a slight increase occurred.

Some selection of dams as well as daughters was indicated by the data. While the daughters could not be selected on actual fleece weight before they were sheared at about one year of age some indirect selection for fleece weight could be practised to a limited extent at weaning time. At weaning time the Corriedale and Rambouillet ewe lambs were scored for body and fleece characteristics and animals deficient in staple length and density were culled out. This was not done with the Romney crossbreds during the years in which data for the present study were collected.

While the correlations between fleece weight and staple length and between fleece weight and density are not high [Spencer *et al.* (16), Malan *et al.* (13), Hill (5), and Lambert *et al.* (10)], they do indicate that culling the individuals most deficient in these characteristics at weaning time would tend to eliminate more of those with the light fleeces than with heavy fleeces. This would lead to a reduction in the variation in the shearling fleece weights of the daughters saved and included in these data.

Some evidence on the amount of selection practised among the dams may be obtained by comparing the mean fleece weight of the sheep used as dams and the mean for the whole population. The mean shearling fleece weight of all dams was 9.17 pounds and that of all shearlings from which the dams were drawn was 8.67 pounds. Thus the mean of the dams was 0.50 pounds above the mean of the population from which they were drawn.

Paternal Half-sib Correlations

An estimate of the year effect, sire effect, and the resemblance between fleece weights of paternal half-sibs was obtained by an analysis of variance as shown in Table 4 for the Canadian Corriedale data, and in a slightly different form for all three groups in Table 5.

The individual variance is that among daughters of the same sire. These differences result partly from genetic differences between paternal half-sibs and partly from environmental factors peculiar to each animal.

TABLE 4.—ANALYSIS OF VARIANCE OF THE FLEECE WEIGHTS OF CANADIAN CORRIEDALE SHEARLING EWES

Source of variation	Degrees of freedom	Sum of squares	Mean square	Interpretation of mean square
Total	172	295.43	1.72	
Between years	4	62.47	15.62**	$1.14 + \frac{173}{20}S + \frac{173}{5}Y$
Within years	168	232.96	1.39	
Between sires within years	15	59.23	3.95**	$1.14 + \frac{173}{20}S$
Within sires within years	153	173.73	1.14	1.14

Paternal half-sib correlation within years $= \frac{1.39 - 1.14}{1.39} = 0.18$

NOTE.—In all analyses of variance in this paper * indicates significance at the 5 per cent level and ** at the 1 per cent level.

The variance due to sire differences is the additional variance caused by differences between sires. That is, individual variance plus variance due to sire differences is the variance that would be found between members of pairs picked at random except that the members of each pair would be born in the same year but sired by different sires. To the extent that differences between sires affected the fleece weights of their daughters the variance within pairs which had different sires would be greater than the variance among daughters by the same sire. Variance due to year differences is the additional variance that would be found between members of daughters picked at random except that members of the pairs would differ in both year and sire.

In the present data the total theoretical variance may be defined as the variance that would occur if all pairs were picked in such a manner that members of each pair were born in different years and sired by different sires.

Strictly speaking this interpretation of the mean square is applicable only to data in which the number of individuals in each group (k) is equal. A correction factor, which compensates for error introduced by unequal group size, has been devised by Winsor and Clarke (21) and this was used in all analyses of this type.

The data for the Rambouillets and Romney crossbreds were analyzed in the same way as the Corriedale data and the results have been brought together in Table 5.

From this table it will be noted that the percentage of the total variance attributable to each source is quite different in the three groups. Especially notable is the relatively low individual variance in the Romney crossbreds and the high proportion of variance attributable to year differences in this group. Contrasted to this is the negligible sire difference.

TABLE 5.—ANALYSIS OF VARIANCE

Source of Variance	Portions of Variance					
	Canadian Corriedale		Rambouillet		Romney Crossbred	
	In actual units	As % of total	In actual units	As % of total	In actual units	As % of total
Year differences	0.34	18.9	0.61	31.6	1.65	56.2
Sire differences within years	0.32	17.8	0.20	10.4	0.06	2.0
Individual variance among paternal sibs	1.14	63.3	1.12	58.0	1.22	41.8
Total	1.80	100.0	1.93	100.0	2.93	100.0
Paternal half-sib correlation within years		0.18		0.14		0.04

Estimates of Heritability of Fleece Weights

The derivation of estimates of heritability from the statistics previously obtained requires consideration of (1) the possible contributions of environmental correlations to the observed resemblance between relatives, (2) whether deviations from random mating have affected the resemblance. By using the path coefficient method devised by Wright (22) the correlation between the phenotype of the dam (P_D) and the phenotype of the offspring (P_O) can be written:

$$rP_DP_O = abh^2g^2 + abh^2g^2m + e'r_{E'E}e = 0.14$$

In the Corriedales the correlation (m) between the genotype of the dam (G_D) and the genotype of the sire (G_S) will have some value greater than zero because some inbreeding was practised in this flock. This inbreeding was mainly half-sib matings but was somewhat irregular and included some sire-daughter matings as well. However, a considerable proportion of the dams came from a random mated flock and the original matings were to sires from the same flock. In these latter matings the correlation would be practically zero. The average value of m in the Corriedale data would have been about 0.2.

The correlation ($r_{E'E}$) between the environment of the dam and the daughter may be assumed to be zero in these data because in a relatively large flock, such as the one from which these data were drawn, special attention in feed and general care cannot be given to individual sheep. Consequently the environment to which all dams and daughters were subjected would be uniform in the sense that there would be no special tendency for the environment of both members of a daughter-dam pair to be above or to be below the flock average for them both.

With $ab = \frac{1}{2}$; $m = 0.2$; and $r_{E'E} = 0$; the equation above becomes $\frac{1}{2}h^2g^2 + \frac{0.2(h^2g^2)}{2} = 0.14$ whence $h^2g^2 = 0.23$.

Likewise it is possible to obtain an estimate of heritability from the paternal half-sib correlation.

In this case the equation is: rP_OP_O, $= a^2b^2h^2g^2 + 3ma^2b^2h^2g^2 + e^2 = 0.18$; $a^2b^2 = \frac{1}{4}$; $m = 0.2$ as before. $e^2 = 0$. Then $\frac{h^2g^2}{4} + \frac{0.6h^2g^2}{4} = 0.18$ and $h^2g^2 = 0.45$.

The environmental term (e^2) is assumed to be zero because the various groups of contemporary half-sibs were raised under similar environmental conditions. All were in the same flock and the various groups were all mixed together as soon as they were born. The situation here, with respect to environmental correlations, is quite different from that existing if data from several flocks had been combined and analyzed as a single population. Under the latter conditions differences in the treatment of the various flocks could lead to an environmental correlation which could be large if fleece weight was much affected by the environmental conditions that varied distinctly from flock to flock.

An additional estimate of the additively hereditary variance may be obtained by simply doubling the intra-sire regression of daughter on dam. This was done and in Table 6, in which the various estimates have been summarized, it may be noted that this estimate is practically the same as the estimate derived from the daughter-dam correlation coefficient.

Estimates of heritability were obtained for the Rambouillets and the Romney crossbreds in the same manner as outlined for the Corriedales. These have been included in Table 6.

Table 6.—Summary of estimates of the additively hereditary variance (h^2g^2) for the three breed groups

Source of estimate	Corriedales	Rambouillets	Romney crossbreds
From daughter-dam regression	0.24	0.40	0.14
From daughter-dam correlation			
$r_{E'E} = 0$ $m = 0$	0.28	0.44	0.14
$r_{E'E} = 0$ $m = 0.2$	0.23	0.37	0.12
From paternal half-sib correlation			
$E = 0$ $m = 0$	0.72	0.56	0.16
$E = 0$ $m = 0.2$	0.45	0.35	0.10

The range of the estimates of the additively hereditary variance is large both between breed groups and within breed groups. Within breed groups the estimate from the paternal half-sib correlation differs more from the other estimates within the Corriedale group than is the case for the other two breed groups. Any error or bias in the half-sib correlation is multiplied by four whereas errors in the parent-offspring resemblance are only doubled. Consequently the estimates based on half-sib resemblance naturally are less reliable (as far as unbiased errors are concerned) than the estimates obtained from the daughter-dam correlations and regressions.

DISCUSSION

The estimates of repeatability of fleece weights obtained in this study were based on unscoured fleeces. Nothing definite is known regarding the repeatability of grease and suint (other normal constituents of fleeces) and variations in these constituents would affect the repeatability of unscoured fleeces.

Certain environmental factors might affect one type of fleece more than another. For example, a relatively long stapled but loose fleece might contain more foreign matter, such as soil, than a short, dense fleece would. The content of this would vary from year to year depending on the climatic and range conditions. Where the year-to-year variation in dust storms was large, the dirt constituent in the unscoured fleece would be expected to be much less repeatable than the clean fibre weight but that need not be so if the variation in fleece type was large and the amount of dust storms was very steady from year to year. Such environmental factors generally would tend to reduce the repeatability of unscoured fleece weights as compared to scoured fleece weights.

The evidence from various sources seems to warrant the conclusion that the repeatability of fleece weights of range sheep will average about 0.5 or slightly over.

The regression coefficients of 0.12 and 0.20, respectively, for the Corriedale and Rambouillet daughters on their dams are not high (0.50 would be expected if heritability were perfect) but indicate that selection may be useful in increasing the fleece weights in succeeding generations. For every increase of a pound in dams' fleeces the increase in daughters' fleeces would be 0.20 pound. This assumes that the sires would be equal to the average of the unselected groups from which the dams were chosen. Selection for the sires would on its own account lead to some improvement of the progeny in addition to that derived from selecting the dams.

The individual variance, in actual units, was practically the same for Corriedales and Rambouillets but the Corriedales showed considerably greater sire differences. This may have been the result of the inbreeding practised in the Corriedale band. The inbreeding had not advanced greatly in the time during which the data had been collected but may have been sufficient to cause some genetic differentiation between lines. If this were true then the average sire differences would include the differences between groups of dams. Such a situation did not apply to either the Rambouillets or Crossbreds. Among them the groups of dams mated to the various sires did not differ systematically from each other.

This same factor of increased genetic resemblance between dams within a line, where some inbreeding was practised, would cause the paternal half-sib correlation to be increased. Therefore, it is believed that the estimate of heritability (h^2g^2) derived from the paternal half-sib correlation is too high for the Corriedales.

For heritability to equal repeatability dominance deviations and epistatic effects must be zero in determining fleece weights and none of the permanent differences between contemporary sheep would have been caused by environmental factors that affected some sheep but not

others (for example, extreme stunting by undernutrition during the suckling period). It seems unlikely that this could be entirely true though it may be that dominance and epistasis are relatively unimportant in their effect on fleece weights.

It appears certain that there is sufficient additively hereditary variance in shearling fleece weights to permit selection to be effective in improving fleece weights in the average range flocks of sheep. The greatest obstacle to extremely rapid improvement will be that the selection differential, i.e., the degree of selection against low fleece weight, cannot be very high. Much of the available freedom to select is used at weaning time when ewe lambs are culled for undesirable characteristics but differences in fleece weight are not recognized with a high degree of accuracy.

Numerous other characteristics will also be considered and this will reduce the intensity of selection for fleece weight. If these other characteristics are correlated positively with fleece weight a little attention to them will not hinder selecting for fleece weight very much but if they are correlated negatively the hindrance will be increased. For example, body weight receives consideration in the selection of replacement ewes and it has been shown (Gartner u. von Ungern-Sternberg (4); Joseph (9); Brody and Campbell (2); and Hunt (6) that body weight and fleece weight are correlated positively to a fairly high degree though some of the evidence indicated that this was not entirely linear. Under certain conditions the largest ewes were poorer wool producers than medium sized ewes. This was related to the optimum size of ewe for range conditions, where the amount of feed was such as to limit the productive ability of larger animals.

There is also evidence, previously cited, that there are positive correlations of varying magnitude between such characteristics as staple length and fleece weight, and density and fleece weight. It may be possible to combine these various characteristics into a compound index such that maximum progress in the improvement of all would be attained. This is a problem to which little attention has yet been given but which is worth further study when data can be obtained.

Another factor placing a limitation on the selection differential, and not subject to very much control, is the relatively low reproductive rate of sheep and the need for a definite number of replacements. Under range conditions ewes usually must be removed from the flock when they reach the age of five years or six years at the most. The reproductive rate is subject to a little control in the matter of rate of multiple births. However, this has practical limits in the economy of sheep production. These limits should be subject of study, especially as related to the effect of twins on selection of replacement stock under range conditions.

The data and analyses presented in this paper lend support to the belief expressed by Hill (5) and Lush and Jones (12) that an important part of the variability in fleece weights is hereditary and that selection can be useful in improving fleece weights both in the generation in which it is practised and in the progeny of that generation. Furthermore, the estimates of hereditary variance obtained in this study are of the same general order as those of Johansson and Berg (7) who estimated the hereditary variance to be not over 35%.

SUMMARY

A study of the inheritance of fleece weights in range sheep was made with data from Canadian Corriedales, Rambouillets, and Romney crossbreds. The mean shearling fleece weights were 8.60 pounds, 7.94 pounds, and 7.13 pounds, respectively, for the Corriedales, Rambouillets, and Romney crossbreds. The standard deviations differed considerably but the coefficient of variation was practically the same for all groups.

For the Corriedales, Rambouillets, and Romney crossbreds, the coefficients of repeatability of fleece weights (among the first three fleeces) were 0.56, 0.56, and 0.43 respectively. These coefficients were of the same order as those obtained by other investigators.

Correlations between the fleece weights of daughters and dams were determined on an intra-year, intra-sire basis. They were: 0.14 for Corriedales, 0.22 for Rambouillets, and 0.07 for Romney crossbreds. The regression of daughters' fleece weights on the fleece weights of dams were 0.13, 0.20, and 0.07 for the three groups in the same order.

Paternal half-sib correlations were obtained as a measure of the resemblance of daughters by the same sire. The correlations were 0.18, 0.14, and 0.04 for Corriedales, Rambouillets, and Romney crossbreds, respectively.

The analysis of variance showed that year differences were highly significant for all breed groups. Sire differences were significant for the Corriedales and Rambouillets, but just below the 5% point for the Romney crossbreds.

Estimates of heritability were deduced from the correlations and regressions. They ranged from a low of 0.14 for the additively hereditary variance in the Romney crossbreds to a high of 0.72 in the Corriedales. The latter figure was probably too high and the maximum would be 0.56, the value of the repeatability coefficient.

The additively hereditary variance is large enough that distinct improvement in fleece weight can be attained through selection.

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STUDIES ON THE EFFECT OF LIQUID AIR, RAYS, AND EMANATIONS ON HONEY YEASTS[1]

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In a previous paper the author (17) has presented a study on the thermal resistance of certain honey yeasts which were found by Lochhead and Farrell (12) to predominate in fermented and unfermented honey. To determine other agents that might be used to kill or to inhibit their growth, yeast inoculated honey was subjected to freezing in liquid air, and exposed to radium, X-ray, shortwave, ultrasonics and ultraviolet irradiation.

REVIEW OF LITERATURE

Liquid Air

Guilliermond (9) cites two workers, Pictet and Young, who submitted yeasts to a temperature of −130° C. (−202° F.) for 24 hours without killing; and one, Doemus, who stated that the yeast of Frohberg could resist temperatures of −150° C. (−238° F.) for 5 to 10 minutes.

Radium

Gager (8) showed that alcoholic fermentation by Fleischmann's yeast was accelerated when 1 gm. of yeast was pressed closely around a glass tube containing radium bromide of 1,500,000 activity for 20 minutes, while Sugiura and Benedict (18) conclude that growth promoting forces in yeast may be inactivated partially by means of exposure to radium emanation. Jacquemin and Giurel (10) found radioactive emanations to have a marked action on alcoholic fermentation and yeasts, exerting a stimulating action from the moment of inoculation of the medium and the growth of the elliptical cells until the final stages. Nadson (14) produced new races from Saccharomyces, Zygosaccharomyces, and other yeasts by the action of radium and X-rays.

X-ray

Th experience of Nadson (14) in producing new races of yeasts from cultures exposed to X-rays elaborates an earlier work when Nadson and Stern (15) found that 2 to 4 minutes' exposure to X-rays produced a change in the vacuole. In 6 to 25 minutes the metachromatic granules coagulated and in 30 to 60 minutes' time death resulted. Fardon, Carroll, and Ruddy (6) reported stimulation of the respiration of yeast suspensions when exposed directly to X-rays.

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[1] Contribution from the Bee Division, Experimental Farms Service, Department of Agriculture, Ottawa, Canada. Abridgment of Parc II of a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture, University of Toronto.

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Short Wave

The literature regarding the lethal effect of Hertzian waves on yeasts is particularly meagre. These radiations, being of radio frequency, are found near the opposite end of the spectrum from the ultraviolet and X-rays.

Ultrasonics

Wave frequencies of 289,000 per second used by Wood and Loomis (20) tore Paramoecium and ruptured the filaments of Spirogyra, and Beckwith and Weaver (3) found that proliferating cultures of *S. ellipsoideus* 2.5 cm. deep in a heavy glass tube with cellophane bottom lowered into a dielectric bath containing the quartz oscillating crystal were usually killed in 15 minutes. Buchanan and Fulmer (4) suggest that the application of these high frequency ultrasonic vibrations opens up a new and fruitful field for biological research.

Ultraviolet

Irradiation with ultraviolet rays results in death in a much shorter time than with X-rays. Thus in the work of Nadson and Stern (15) cultures of *Saccharomyces cerevisiae* were killed in less than 2 minutes' exposure to ultraviolet, whereas it required between 30 to 60 minutes with X-rays. The cells of *S. Ludwigii* were more susceptible. The authors also report that short time exposure of the medium to ultraviolet produced no effect on the subsequently grown cells, but exposure of 7 hours and 15 minutes to a mercury vapour lamp was found by Bailey, Woodrow, and Fulmer (1) practically to inhibit yeast growth.

Fardon, Carroll and Ruddy (6) found stimulation to be quite similar when yeast suspensions were irradiated with ultraviolet and X-rays, while Beckwith and Donovick (2) found an initial depression in the formation of ethyl alcohol when a pure culture of yeast was treated with ultraviolet. This was followed by increased fermenting activity which was maintained thereafter.

Owen and Mobley (16) determined that exposure of yeast to rays of 2,300 to 3,100 Angstrom units for 1 minute gave greatest stimulation in cell development, while 3 minutes' exposure retarded the rate of development. Using a bottom yeast Lindner (11) found that the velocity of fermentation of dextrose was greatly increased by exposure to ultraviolet rays, but exposure in a shallow layer produced fatal results.

Fazi (7) observed great resistance of brewer's yeast to ultraviolet of 1,200 candle power at 20 cm., although Tanner and Ryder (19) discredit this, proving that yeast cells possess no marked resistance to ultraviolet rays. When cultures were exposed in water 8 mm. in depth to a 1,200 c.p. Cooper-Hewitt lamp operated at 110 volts and 4 amperes (D.C.) at a distance of 20 cm. the survival time was not more than 10 minutes. Buchta (5) found that cells of *S. cerevisiae* and *S. Ludwigii* could not withstand more than a 3 minute exposure to ultraviolet rays. Loofbourow and Cameron (13) determined that the lethal action on *S. cerevisiae* began abruptly at a wave length of 2,900 Angstrom units.

EXPERIMENTAL WORK

The purpose of the investigation was to determine if there was any method other than the application of heat that might be used for the destruction or inhibition of yeasts causing honey fermentation.

The yeasts used are designated as follows:

M1 — *Zygosaccharomyces richteri* Lochhead & Heron

J7 — *Zygosaccharomyces nussbaumeri* Lochhead & Heron

138 — *Zygosaccharomyces priorianus* Klöcker (isolated by Lochhead & Farrell)

139 — *Zygosaccharomyces rugosus* Lochhead and Farrell

In the experiment with liquid air species E6, a yeast isolated by Lochhead and Heron and classified as *Zygosaccharomyces barkeri* was used.

MEDIA EMPLOYED

Thirty Per Cent Honey Agar

Peptone	1.0 gm. per litre
Potassium diphosphate	1.0 gm. per litre
Magnesium sulphate	0.5 gm. per litre
Ammonium tartrate	0.5 gm. per litre
Sodium chloride	0.1 gm. per litre
Calcium chloride	0.1 gm. per litre
Agar	25.0 gm. per litre
Honey	300.0 gm. per litre

2 : 1 Honey Nutrient Solution

2 Parts of honey by weight were added to 1 part of nutrient solution containing the salts and peptone as used in the preparation of honey agar.

50 : 50 Dilution Blanks

Equal parts by weight of honey and water.

PROCEDURE

Slant cultures of the yeast on 15% honey agar, incubated for 2 weeks at room temperature, were washed with 2 : 1 nutrient solution. Care was taken to brush into suspension with the platinum needle only those cells from the moist medium. After incubation for 24 hr. at 30° C. (86° F.) this suspension was used to inoculate sterile honey.

Where possible inoculated honey was exposed directly in petri dishes and also in plates poured with 30% honey agar. The former contained 10 ml., the latter 1 ml. of the inoculated honey. The depth in each case was about 1.5 mm.

Following exposure, the agar plates were placed in the incubator and 1 ml. was taken from the exposed honey and routine quantitative and qualitative tests made for growth and fermentation, incubation being at 30° C. (86° F.).

Check agar plates were made in serial dilution in order to get an estimate of the number of yeasts per ml.

Liquid Air

Honey previously inoculated with yeast E6 and having a count of 4,000 per gram was poured into aluminum centrifuge tubes. Duplicate tubes were lowered into a flask containing liquid air at −190° C. (−310° F.) for a period of 5 min. It was then removed and the honey allowed to come to room temperature. Samples of the honey were taken for testing and the remainder was again frozen in the liquid air for a period of 10 min. When removed and warmed to room temperature, samples were taken again. Yeasts M1, J7, 138, and 139 were not used for this test.

Radium

A 10 mg. needle was placed on top of the plates containing the honey and the agar.

X-ray

Agar plates were held on edge and exposed, at a distance of 18 in. from the target, to X-rays travelling horizontally. One half of each uncovered plate was shielded by a sheet of lead $\frac{1}{8}$ in. in thickness. Uncovered honey plates, about 6 in. beneath the tube, were exposed to the indirect rays for the same length of time as the agar plates.

The X-ray machine operated on a voltage of 95,000 and drew a current of 2 milliamperes. The tube was of the Universal Coolidge type with tungsten target, emitting waves of 0.14 Angstrom units in length.

Short Wave

The tubes containing 10 ml. of honey were set between the plates of a machine whose wave length was 25 m., operating at a frequency of 12 megacycles per sec. The agar plates and honey plates were also placed in the path of the current between the two plates.

Ultrasonics

In using the ultrasonic instrument, it was only possible to use honey in test tubes. Trial was made, using varying amounts of honey.

The instrument had a quartz crystal, the frequency of vibration of which was 427 kilocycles per sec.

Ultraviolet

(a) Laboratory demonstration lamp. The uncovered petri dishes were set as close to the source of light as possible, two at a time. The radiating fins made it impossible to set the plates nearer than about $4\frac{1}{2}$ in. and shaded part of the plates.

The lamp was of the Cooper-Hewitt type and drew a current varying from 2 to 2.2 amperes at voltage varying from 62 to 65.5. The set-up was such as is used for ordinary laboratory demonstration purposes and had a quartz tube about 4 in. in length.

(b) Sterilamp. A "Sterilamp", the product of the Westinghouse Electric and Manufacturing Company, Bloomfield, N.J. (described in publication A-2793 n.d.), was installed in the laboratory by a representative of the Company. This "lamp" is especially designed for maximum effect in the region from 2,000 to 2,800 Angstrom units. This is the region found to be the most lethal in the treatment of bacteria. The set-up consisted of a transformer which gave a voltage of 375, and a 13 watt "lamp" which drew a current of 0.035 amperes.

The honey was poured over a pane of glass, set at an angle of 23° to the horizontal, with the middle about 2 in. below the "Sterilamp". In order to facilitate the flow of honey, two 60 watt bulbs were placed in the supporting box to warm the glass.

RESULTS

Liquid Air

Plates poured from the honey inoculated with culture E6 showed no signs of killing as a result of being frozen for 5 and 10 minutes in liquid air at −190° C. (−310° F.). This is obvious from Table 1. Tubes corresponding to the plate cultures showed vigorous fermentation at the end of 1 week.

TABLE 1.—SURVIVORS OF A SPECIES OF ZYGOSACCHAROMYCES FROZEN IN LIQUID AIR AT −190° C. (−310° F.)

Tubes	Checks	5 min.	10 min.
1	3,150 4,200	3,900 4,050	4,800 4,950
2	4,500 4,200	3,750 3,750	4,200 3,900

Radium

Neither plates poured with the honey, nor the original agar plates, showed any sign of killing after exposure ranging from 40 minutes to 1 hour. These results are apparent from Table 2. Corresponding tubes showed vigorous fermentation after 1 week's incubation. Culture J7 was not treated in this test.

TABLE 2.—SURVIVORS OF ZYGOSACCHAROMYCES SPECIES IN HONEY AND AGAR EXPOSED TO RADIUM

Species	Checks	Exposure	Honey	Agar
M1	11,800 13,500	40 min.	15,500 16,250	No killing No killing
138	24,500 23,600	60 min.	21,000 22,250	No killing No killing
139	15,400 17,000	60 min.	13,000 13,500	No killing No killing

X-ray

Table 3 shows the results obtained after exposing honey plates to the influence of X-rays for a period of time. Agar plates exposed showed no evidence of killing, since it was impossible to detect which half of the plate had been exposed and which half had been covered by the $\frac{1}{8}$ in. lead plate.

In order to determine whether culture 139, which showed no growth on the plates poured from the exposed honey, had been killed by exposure, a second test was made, exposing the honey plates for periods of 5 and 10 min. All tests made of this honey proved it to be sterile, with the exception of one plate poured from the honey exposed for 10 min. This one had three yeasts growing on it. It is thought that the original inoculum may have been at fault, since the check plates for the second test showed no signs of growth either.

TABLE 3.—SURVIVORS OF ZYGOSACCHAROMYCES SPECIES IN HONEY AND AGAR EXPOSED TO X-RAY

Species	Checks	Exposure	Honey	Agar
M1	11,800	50 min.	1,990	No killing
	13,500		1,500	No killing
J7	8,000	30 min.	11,950	No killing
	5,200		10,800	No killing
138	24,500	30 min.	19,800	No killing
	23,600		17,250	No killing

Short Wave

Agar plates set in the path of the current between the plates showed condensation on the lids in 5 to 50 seconds' time and were very warm in 1 min., the agar being liquefied, at which time they were removed.

The plates containing honey were noticeably warm in 1 min., condensation on the lids being apparent. Since the greatest amount of electricity would pass through the plates diametrically at right angles to the plates of the machine, this course was marked and, after treatment, the honey was poured off in such a way as to include most of it that had been in the path of the current.

The test tubes containing inoculum from each of the four species were set side by side in a line at right angles to the plates, so that they would form a more or less continuous path for the current. The tubes remained in the machine for 20 min., during which time the temperature of the honey rose from 29° to 41° C. (84.2° to 105.8° F.). Table 4 shows the results obtained.

The decrease in the yeast count at the 10 min. period, in the case of the honey exposed in the plates, was caused, in all probability, by the increase in the temperature, as the plates, when removed, were too hot to hold in the hand. Table 4 also shows the effect of the current on the yeasts in the agar.

TABLE 4.—SURVIVORS OF ZYGOSACCHAROMYCES SPECIES IN HONEY AND AGAR EXPOSED TO SHORT WAVE

Species	Checks	Honey			Agar
		Plates		Tubes	1 min.
		5 min.	10 min.	20 min.	
M1	11,800 13,500	9,000 10,000	3,390 3,030	9,400 9,200	Some at one side
J7	8,000 5,200	16,800 14,250	1,260 1,220	17,800 17,600	Some at each side
138	24,500 23,600	3,650 4,340	3,770 3,386	15,600 17,700	All killed
139	15,400 17,000	6,900 7,500	650 550	8,200 8,300	Some at each side

Ultrasonics

In preliminary tests, it was observed that the temperature of the small sample of honey rose quite rapidly. Varying amounts were used and the time also varied. These appear in Table 5 along with the results.

TABLE 5.—TIME, TEMPERATURE-RANGE, AND AMOUNT OF HONEY INOCULATED WITH SPECIES OF ZYGOSACCHAROMYCES TREATED WITH ULTRASONICS

Species	Check	Test	Time	Temp. range	Amount	Count per ml.
M1	10,650	1	90 sec.	25 – 56° C. 77 – 132.8° F.	2¾ ml.	3,075
	10,650	2	60 sec.	31 – 49° C. 87.8 – 120.2° F.	2¾ ml.	6,525
	10,650	3	30 sec.	33 – 45° C. 91.4 – 113.2° F.	3 ml.	8,775
J7	18,900	1	10 min.	15 – 75° C. 59 – 167° F.	10 ml.	Nil
	18,900	2	60 sec.	31 – 58° C. 87.8 – 136.4° F.	2½ ml.	8,475
138	22,700	1	45 sec.	33 – 51° C. 91.4 – 123.8° F.	2¼ ml.	4,050
	22,700	2	60 sec.	25 – 53° C. 77 – 127.4° F.	3 ml.	8,925
139	10,550	1	90 sec.	20 – 45° C. 68 – 113° F.	2½ ml.	3
	10,550	2	60 sec.	25 – 50° C. 77 – 122° F.	3¼ ml.	4,575

Ultraviolet

(a) Laboratory demonstration lamp. From Table 6 it may be observed that ultraviolet has lethal effect on yeast cells when exposed in agar and in honey. However, the length of time required would seem to place it beyond use on an extensive scale.

TABLE 6.—SURVIVORS OF ZYGOSACCHAROMYCES SPECIES IN HONEY AND AGAR EXPOSED TO ULTRAVIOLET

Species	Check	Exposure	Honey	Agar
M1	11,800	60 min.*	18,750	Killed below light
	13,500	60 min.	3,500	Slight killing
J7	8,000	60 min.*	4,065	Heaviest killing
	5,200	60 min.	4,170	Killed below light
138	24,500	60 min.*	4,535	Heavy killing
	23,600	60 min.	7,245	Killed below light
139	15,400	60 min.*	4,520	Killed below light
	17,000	60 min.	2,015	Killed below light

* Because of the discontinuity of light, this time was reduced considerably in the first test with each species.

(b) Sterilamp. For "Sterilamp" tests, 0.1 ml. of inoculum was put into 5 ml. 2:1 solution and the whole stirred into 70 ml. sterile honey, which was poured over the slightly heated sloping glass beneath the "Sterilamp".

A thermometer lying on the glass registered 60° C. (140° F.), but when the honey was poured over the glass, the thermometer gradually dropped to 42° C. (107.6° F.). The length of pouring time was approximately 2 min. Forty-five seconds after starting to pour, honey was running off the lower edge of glass. In 3 min., the honey was nearly all run off. The remaining honey was scraped off with a glass rod.

It is evident from the results presented in Table 7 that the short period of time required to run honey over the glass (approximately 45 sec.) was not sufficient time of exposure to allow the ultraviolet rays from the "Sterilamp" to penetrate the thin film of honey and kill the yeast cells. The results include some of the first run off, "B", some poured from the tray, "C", and some of the last scraped off, "D". Since 139 control plates were sterile, the results for this species are not included in the table.

TABLE 7.—SURVIVORS OF ZYGOSACCHAROMYCES SPECIES IN HONEY IRRADIATED WITH "STERILAMP" EMANATIONS

Species	A Check	B Run off	C From tray	D Scraped off
M1	25,100	9,600	5,500	5,000
	28,500	14,000	6,000	6,000
J7	26,200	38,500	4,400	6,000
	23,400	48,500	4,480	6,900
138	13,900	4,890	3,190	2,840
	12,400	5,490	3,190	2,510

CONCLUSION

Freezing an inoculated sample of honey in liquid air at −190° C. (−310° F.) for two periods totalling 15 min. had no apparent effect on the inoculum, culture E6.

The application of radium for 1 hr. had no lethal effect on honey yeasts suspended in honey or in agar.

Exposure of honey yeasts in agar at 18 in. to X-rays of 0.14 Angstrom units gave no evidence of killing. Some killing may have resulted when honey was exposed in plates 6 in. below the target.

Hertzian waves of 2.5×10^1 Angstrom units and frequency of 12 megacycles per sec. caused death to yeast cultures in agar in plates set between the plates of a shortwave machine for 1 min. Lethal effect was evident when inoculated honey was placed in petri dishes in the path of the current. When a series of test tubes containing the inoculated honey was set at right angles to the plates for 20 min. some killing was observed. However, there was a constant increase in temperature, a fact that no doubt contributed to the lethal effect of the waves when applied to the petri dish cultures.

Sound waves of a frequency of 427,000 cycles per sec. caused increase in temperature when an ordinary test tube containing inoculated honey was lowered into the oil bath of the ultrasonic instrument. The rise in temperature was dependent upon the amount of honey and the length of time the tube remained in the bath, but it was sufficient in most cases to cause killing of the yeasts used to inoculate the honey. Some killing may have resulted from the action of the waves themselves.

There was appreciable killing when the inoculated honey and the agar plates were exposed to ultraviolet. The greater lethal effect was on the yeast cultures exposed in agar. When a special source of ultraviolet was used giving out rays which have been found to be particularly effective in destroying micro-organisms, there was considerable killing, even though the time of exposure was only about 3 min. Growth of the yeasts following treatment also may have been inhibited because of the exposure of the honey. Since the effectiveness of light rays varies inversely with the square of the distance from the source, it is felt that by applying the "Sterilamp" emanations more closely the combination of the lethal effect on the yeasts and the possible inhibiting effect of the exposed honey, that subsequent fermentation might be controlled.

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PERENNIAL SOW THISTLE AND ITS SMOOTH VARIETY IN CANADA[1]

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The perennial sow thistle of Eastern Canada is *Sonchus arvensis* L., a glandular hairy species which occurs only sparingly west of Lake Superior. The sow thistle westward is a smooth variety of the above, var. *glabrescens* Guenth., Grab., and Wimm., which occurs only sparingly in the East. The latter invasion commenced in Manitoba nearly 50 years ago, some 30 years after the former had begun to attract attention in the East (3).

From weed survey records, supported only to a limited extent by collections, Table 1 has been prepared to present in more detail the incidence of each sow thistle from coast to coast. Each figure for percentage represents the number of lists, of 100 secured, in which the weed was recorded. In the last column the figures are reduced to their more easily comparable ratios. The various regional break-downs have been fixed for smoothness of result in the course of the study of the data.

TABLE 1.—PERCENTAGE INCIDENCE OF *Sonchus arvensis* AND ITS VAR. *glabrescens* IN CANADA

Longitude	No. of surveys	*S. arvensis*		var. *glabrescens*		Ratio
		No.	%	No.	%	
Canada	4315	(1057)	24.5	(536)	12.4	2–1
60°–88° (Eastern Canada)	2805	(952)	33.9	(71)	2.5	13.5–1
89°–131° (Western Canada)	1510	(105)	7	(465)	30.8	1–4.4
Eastern Canada						
60°–67° (Maritimes-Gaspé)	1005	(271)	27	(15)	1.4	19.3–1
68°–82° (Quebec-Ontario, E. of L. Huron)	1723	(667)	38.7	(47)	2.7	14.3–1
83°–88° (L. Superior region)	77	(14)	18.2	(9)	11.7	1.5–1
Western Canada						
89°–94° (Northwestern Ontario)	72	(13)	18	(38)	52.8	1–2.9
95°–115° (Prairie Provinces)	796	(42)	5.3	(364)	45.7	1–8.6
116°–131° (Peace River-British Columbia	642	(50)	7.7	(63)	9.8	1–1.3
Prairie Provinces						
95°–101° (Manitoba)	199	(5)	2.5	(152)	76.4	1–30.5
102°–109° (Saskatchewan)	326	(22)	6.7	(156)	47.8	1–7.1
110°–115° (Alberta E. of 116°)	271	(15)	5.5	(57)	21	1–4
116°–119° (Alberta–B.C., E. of 120°)	279	(23)	8.2	(44)	15.7	1–1.9

DISCUSSION

The ratio of 2–1 between the two sow thistles for Canada may be considered alongside a ratio of 3–1 obtained when 105 specimens, 87 eastern

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and 18 western, were examined. Of these 79 were found to be *S. arvensis* and 26 the variety. These ratios might be narrowed, or even reversed, if West and East were more equally represented. There are roughly five eastern specimens to one western, and two eastern surveys to one western, as East and West have been delimited. The narrower ratio from the numerous well-distributed surveys may well be the more nearly correct.

Separate analysis of eastern and western records establishes distinct preponderance of the species in the East and of the variety in the West. Again the ratios between the two, 13.5–1 in Eastern Canada and 1–4.4 in the West, may be considered alongside those from specimens. For the East 72 specimens of *S. arvensis* to 15 of the variety gives a ratio of 4.8–1; for the West 7 of the former to 11 of the latter, about 1–1.6. If it is a fair assumption that casual collecting, by which most of these specimens were secured, tends to be of the "novel" or less prevalent type, their eastern and western ratios might be expected to differ, in actual fact, more nearly in accord with those obtained from surveys. Systematic collecting carried out in 1939 in one considerable area (Eastern Ontario) supports this assumption inasmuch as it yields the wider ratio of 12–1, nearly that from surveys in Eastern Canada.

Localization of the above reverse of incidence is found in the neighbourhood of Port Arthur, Ont., which accordingly may be taken to divide East from West. The slightly inverse ratio of 1–2.9 in northwestern Ontario, from 1.5–1 in the Lake Superior region, arises almost entirely from a sharp increase there of var. *glabrescens*. At this point wind-borne spread from either direction may be supposed to be largely inhibited by the tremendous distances of virtual wilderness. Railway traffic would be the chief means of transit and movement of western grain to trans-shipment at the lake head would bring with it an excess of the variety to offset a lighter but longer continued dispersal of the species from the East.

For Eastern Canada the ratios show a diminishing trend westward to near parity as already found. The inverse ratios for Western Canada leave near parity behind in northwestern Ontario only to approach it again west of Long. 116 in western Alberta. Between these points the low incidence of *S. arvensis* reaches its extreme low of 2.5 in Manitoba just where the high, for the West, of the variety reaches its peak of 76.4%, and a ratio between them of 1–30.5. Incidences for *S. arvensis* show little of trend in either East or West to indicate any direction of present active spread. Those for the variety diminish smoothly both eastward and westward from Manitoba, suggesting this as the initial plague spot for invasions still in progress into Peace River and British Columbia frontiers and possibly eastward. Both sow thistles apparently reached Manitoba during the nineties of last century: a Macoun specimen in the National Herbarium collected at Brandon in 1896 is *S. arvensis*, and there is good evidence for the presence of the variety at about the same date. With practically an even start in Manitoba, and a lead in Canada of another 30 years or more, the movements of *S. arvensis* seem to require other explanation than is afforded by the factor of time alone. Likewise, the variety, with equal time for spread eastward and westward, al owance being made for the northern Ontario barrier, has yet made inferior progress into the East.

The inference would be that environmental factors enter into the question. Eastern and probably British Columbia conditions, as opposed to prairie conditions, might be suspected of holding the solution if two so closely allied plants could be expected to differ so fundamentally. The meeting place of encroachment, however, has been shown to be not at the transition to prairie but at the lake head. Is there any other demarcation available then, which will fit the case?

In view of a generally recognized partiality for moisture (on the part of both, however), the factor of aridity has been examined. Use was made of a mapping presented by Hurd and Grindley (2) in which temperature and rainfall in the prairie provinces during the months of June and July provide a quotient for delimiting zones of aridity greater, and less than, mean. As shown in Table 2, the comparatively few records of *S. arvensis*, and the more numerous records of the variety are both more abundant outside the zone of high aridity. Their ratios indicate almost identical response to aridity, offering no explanation of the disparity between them shown especially in Manitoba.

TABLE 2.—PERCENTAGE INCIDENCE IN RELATION TO ARIDITY

Prairie provinces (E. of 117° and Rocky Mts.)—820 surveys

	Surveys recording	More than mean		Less than mean		Ratio
			%		%	
S. arvensis	46	(9)	4.1	(37)	6.1	1 – 1.5
var. *glabrescens*	375	(66)	30.4	(309)	51	1 – 1.6

For another attack, on the basis of soils, use was made of a generalized soil map prepared in the Field Husbandry Division, Central Experimental Farm, Ottawa (1). The brown and dark brown soils of Saskatchewan and Alberta were opposed to the black soils which prevail in Manitoba and extend westward to the north of the others. To quite an extent this delimitation coincides with that for aridity, and such realignment of data as was involved has served chiefly to emphasize what was already found. Both sow thistles have higher incidence on the black soils. Their ratios again differ too little to suggest any appreciable unlikeness of habit. Not until Manitoba, or that part of it characterized by an extreme of the black

TABLE 3.—PERCENTAGE INCIDENCE IN RELATION TO SOILS

Prairie provinces (96° – 114°)—664 surveys

	Surveys in which recorded	Brown and dark brown soils		Black soils		Ratio
			%		%	
S. arvensis	35	(15)	4.9	(20)	5.5	1 – 1.1
var. *glabrescens*	296	(96)	31.2	(200)	55.5	1 – 1.8

soils in the Red River valley, is taken as a contrasting unit, does contrasting response become marked. What, if anything, this soil would have to contribute for the variety, which is inhibitive to the species, is not apparent.

Sow thistle is generally regarded as partial to somewhat basic, though not highly alkaline, soil reactions. In Nova Scotia, a distinction is sometimes made between a more and a less acid environment south and north of a line roughly from Digby to east of Halifax, and again between the southeastern and remaining portions of Cape Breton. *S. arvensis* was shown by the surveys, and in even greater extreme by an unpublished map seen through the courtesy of Prof. A. E. Roland, to have an incidence from two to four times greater on the more basic soils. Prof. Roland's map of twelve collections of the variety showing agreement, with a 3–1 ratio, is accepted in the absence of enough records from the surveys. Until similar comparisons can be made elsewhere the indications are, therefore, that both sow thistles alike flourish best on the more basic soils.

Other factors, as cropping or other agricultural practices, might be analysed if they were more clear-cut. For the present the extremely wide ratio between the two sow thistles in Manitoba and their inverse ratios as between East and West must remain without ecological interpretation. That the plants actually differ in more than name, and presence or absence of glandular hairs, is recognizable botanically and, when growing together, without difficulty. Colonies in close proximity at Murray Bay, Que., although at a similar stage of development, differed in height from about three feet in the case of the species to five and six feet in the case of the variety, which also had broader, less deeply lobed leaves. The percentage of developed seed was much less for the variety. May this vegetative vigour have a bearing on the intensity of the sow thistle problem in parts of the West?

Citations of the 79 specimens of *S. arvensis* would show distribution from Cape Breton to Vancouver Island, including one from James Bay. The 26 specimens of var. *glabrescens* range from New Brunswick to Prince Rupert, B.C., with one from Waterways in northern Alberta. With distributional and ecological evidence so largely negative the striking incidences found can only be attributed then, provisionally at least, to fortuitous determinants such as advent and natural barriers.

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CONTRIBUTIONS TO THE STUDY OF RANCIDITY IN CANADIAN CHEDDAR CHEESE

I. BUTYRIC ACID CLOSTRIDIA IN RANCID CHEESE

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Rancidity in Canadian cheddar cheese remains a serious and unsolved problem. As Canada continues to increase her cheese production to meet the war-time requirements of Great Britain this problem becomes increasingly important. It is also becoming evident that a thorough understanding of the cause and control of rancidity in cheese may yield information helpful in the control of other types of spoilage in dairy products.

In a recent address before the Central Ontario Cheesemaker's Association, Hood (2) gave an outline of the difficulties involved in solving this problem of rancidity, and indicated some of the experimental work that has been, and is being done towards its solution. The two chief factors suspected of causing cheese to become rancid are the butyric-acid-forming bacteria and the enzyme, milk lipase. By studing the effect of adding pancreatic lipase to milk for cheesemaking, as well as the increasing knowledge of the milk enzyme itself, the general tendency is to believe that the causal agent is of enzymatic rather than of bacterial origin. However, up to the present time, no clear cut experiments have been produced to show that either of these factors are the sole cause of the characteristic butyric acid flavour which develops in cheese under factory conditions. Our growing knowledge of the lipase enzyme itself, and of the many factors affecting its activity, suggest that the solution is to be looked for in a combination of causes rather than in any single, simple explanation.

The present studies consist of a series of observations and experiments extending over a period of several years and dealing with various phases of the subject. Some of the experiments have yielded definitely negative results; but as such, it is felt that they are of sufficient value to be recorded.

I. BUTYRIC ACID CLOSTRIDIA IN RANCID CHEESE

This portion of the work consists of an attempt to isolate and enumerate the butyric acid anaerobes in rancid and normal cheese. This is followed by Part II in which attempts are made to follow the growth and activity of clostridia purposely inoculated into the cheese under laboratory conditions.

Preliminary direct microscopical examination of 40 rancid cheese failed to show typical clostridial or plectridial forms. They were, however, frequently observed when low dilutions (1 : 100) of cheese were made in sterile water and incubated at room temperature. It was also noted that the addition of small amounts of ammonium hydroxide hastened sporulation

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of these cell types. This is well illustrated by one set of observations made on a group of 7 rancid cheese, as shown in Table 1. Approximately one gram of each cheese was put into 100 ml. of sterile water. After crushing and vigorously shaking, each was divided into 2 portions, and 2 drops of concentrated ammonium hydroxide were added to each sample of one set. These were examined daily under the microscope for the presence of typical plectridia or clostridia. The seeming decrease of clostridial cells in the suspensions where NH_4OH had been added, after 7 days incubation, simply means that the vegetative portion of the cells had disintegrated, leaving spores without any identifiable characteristics.

TABLE 1.—THE DEVELOPMENT OF CLOSTRIDIAL TYPES OF CELLS IN RANCID CHEESE SUSPENSIONS, WITH AND WITHOUT ADDED AMMONIUM HYDROXIDE AS OBSERVED UNDER THE MICROSCOPE

No.	At 24 hours		At 3 days		At 7 days	
	+ NH_4OH	− NH_4OH	+ NH_4OH	− NH_4OH	+ NH_4OH	− NH_4OH
1	−	−	+++	+	−	++
2	+	−	++	−	−	++
3	−	−	−	−	+	+
4	−	−	+	−	−	+++
5	−	−	+	−	+	++
6	−	−	?	−	−	++
7	−	−	+	−	+	++

The number of +'s indicates the relative abundance of typical cells; − indicate none were observed.

TABLE 2.—THE DEVELOPMENT OF CLOSTRIDIAL AND PLECTRIDIAL TYPES IN SUSPENSIONS OF RANCID AND NORMAL CHEESE, AS OBSERVED UNDER THE MICROSCOPE

Cheese	At 5 days		At 35 days		Cell types
	+ NH_4OH	− NH_4OH	+ NH_4OH	− NH_4OH	
1. Rancid	++	−	?	++	Clost. and plect.
2. Rancid	++	−	?	++	Clostridia
3. Non-rancid	−	−	−	−	−
4. Non-rancid	−	−	−	+	Plectridia
5. Non-rancid	−	−	?	++	Clost. and plect.
6. Non-rancid	−	−	+	++	Plectridia
7. Non-rancid	−	−	?	−	−

TABLE 3.—THE HIGHEST DILUTIONS OF 47 RANCID AND SLIGHTLY RANCID AND 17 NON RANCID CHEESE YIELDING TYPICAL IODOPHILIC CLOSTRIDIA

Type	None present	1^{-1}	1^{-2}	1^{-3}	1^{-4}	1^{-5}	1^{-6}	1^{-7}
Rancid	9	3	15	8	5	3	4	0
Non-rancid	7	4	3	3	0	0	0	0

It was also observed that suspensions of many of the non-rancid samples, if left long enough would also develop these characteristic spore types. More frequently the non-rancid samples developed the long thin plectridial types, rather than typical clostridia. Table 2 gives the microscopical observations on 5 non-rancid and 2 rancid samples of cheese at 5 and 35 days after being suspended in sterile water. In almost every instance, the definitely rancid cheese developed these characteristic clostridial cells much sooner and more abundantly than the "slightly rancid" and non-rancid samples. These spore types were never observed where the cheese was diluted more than 1 in 10,000.

Enrichment media. Three types of enrichment media were used for culturing the butyric anaerobes from cheese: corn-liver medium of McClung and McCoy (5), a grass medium (1), and a medium composed of malt extract syrup 5%, proteose peptone 0.2%, $CaCO_3$ 2%. The usual procedure was to make dilutions of the cheese in sterile water as high as 1-^{7} and to transfer 1 ml. of the suspension from each dilution to one or more tubes of enrichment medium. These were incubated for 5 days at 37° C., pasteurized at 85° C. for 10 minutes and reincubated for at least 7 days. Frequently, after pasteurization, transfers were made to fresh tubes of media. The presence of vigorous or frothy fermentation, accompanied by large spindle shaped clostridia, giving a typical granulose reaction with iodine was considered as indicative of organisms of the butyric acid group. Table 3 gives the results of these tests with 47 rancid and slightly rancid cheese and 17 non-rancid samples. The samples in which clostridia were found present in the 1^{-5} and 1^{-6} dilutions had in every case been graded as "rancid." It was also interesting to observe that 2 of the non-rancid samples containing clostridia in 1^{-3} dilution were from factories with previous cases of rancidity.

Several samples of real "stinker" types of cheese were also sent in for examination. Invariably these samples had millions of clostridia per gram of cheese.

Although not bearing directly on the question of butyric anaerobes, another observation seems worthy of mention. This is the difference in the general microflora of the rancid and non-rancid cheese as observed by microscopical observation. In general the various samples of rancid cheese did not appear to have a common or a characteristic microflora. But they did differ from most normal cheese in having a wider variety of organisms, especially of rod-shaped cells. The normal cheese not only had a narrower range of cell types but also relatively larger numbers of typical lactic short chain streptococci and diplococci.

DISCUSSION

When one considers the relative abundance of the butyric anaerobes in manure, soil, water, silage, etc., it seems remarkable that cheese has not more of these organisms than the results here would indicate. Hussong and Hammer (4), Rodella (6), Wolf (7) and many others have found at least small numbers of these organisms in almost all samples of milk or cream examined. And unfortunately there is little to suggest that milk for cheesemaking would be an exception to this rule.

When one further considers the numbers of bacteria necessary to bring about a perceptible change in other types of food spoilage, the figures quoted here have little real significance. As shown by Hammer (3), from 30 to 90 million cells of *S. lactis* are required to bring about a recognizable increase in the acid flavour of milk, and from 10 to 66 millions of cells of another organism to produce a bitter flavour.

On the other hand the iodophilic clostridia were more prevalent in the rancid than in the non-rancid cheese. This may be owing to greater initial contamination, or it may be that the mechanism for the suppression of the butyric anaerobes in cheese is less effective where rancidity has occurred.

SUMMARY

Iodophilic clostridia have been shown to be more prevalent in the rancid than in the non-rancid cheese. The number of these organisms found present in rancid samples, however, was not considered sufficient to be a significant factor in the production of rancid flavours.

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